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## REPRODUCTION WITHOUT MALES IN ASEPTIC ROOT CULTURES OF THE ROOT-KNOT NEMATODE[1]


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Parthenogenesis and syngonism[3] are recognized phenomena among the Nematoda, yet in the root-knot nematode, *Heterodera marioni* (Cornu),[4] where males do occur, their function in reproduction has been taken for granted. Byars (1914), however, did suggest the possibility of parthenogenesis in this nematode. Gabriel (1926) assumed its probability because he found several generations in one gall.


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[3] Maupas (1900), Cobb (1918), and others, have found that in certain free-living nematodes the gonad of the female produces spermatozoa, which are stored in a seminal receptacle and fertilize the eggs which are produced subsequently.

[4] This nematode has long been known as *Heterodera radicicola;* but, as Goodey (1932) points out, the original description of *Anguillula radicicola* by Greeff (1872) obviously does not refer to the root-knot nematode. Cornu (1879), then, was the first to publish a name for the latter, which he called *Anguillula marioni.* His descriptions and figures, though inaccurate and incomplete, do apply unmistakably to the root-knot nematode.

The genus (or subgenus) *Caconema,* proposed by Cobb (1924), is founded on three points: the type of parasitism, the structure of the amphids, and the number of testes. None of these is a distinguishing generic character in the classification of nematodes. Plant-parasitic and free-living species are commonly contained within the same genus (Imperial Bureau of Agricultural Parasitology, 1932), e.g., *Anguillulina* and *Pathoaphelenchus* [now *Aphelenchoides* (Steiner, 1932)]. The amphids vary in position, size, and structure from species to species (Steiner, 1923). The reproductive organs also are highly variable, as was observed by Bütschli in 1874. According to Baylis and Daubney (1926), each of the following genera contains species with two testes and species with only one: *Diplogaster, Tylencholaimus, Monhystera, Oncholaimus, Linhomoeus, Cyatholaimus, Chromadora,* and *Laxus,* a genus "of doubtful status," in which Cobb (1894; 1914) himself combined the two types of males. That Baylis and Daubney do not consider the number of testes a significant taxonomic character is indicated by the fact that they make no mention of the point in their synopsis of many of the genera of Ascaroidea, including *Spilophora,* which may be added to the list on the authority of Bütschli (1874). In the descriptions for the other four orders, testes are mentioned for only one genus.

Reproduction without males is obviously a great advantage to a parasite which must develop and lay eggs in one isolated location. There is, however, no true evidence for the phenomenon without actual pedigrees of isolated females, for which controlled laboratory cultivation is necessary.

Berliner and Busch (1914) made preliminary attempts to raise the sugar-beet nematode, *Heterodera schachtii*, in seedlings in agar. Byars (1914) raised the root-knot nematode aseptically through one generation in seedling cultures.

## METHOD

The method of cultivation described in the present paper permits the easy observation of individual nematodes in a fairly healthy environment. Complicating factors are eliminated by keeping the cultures bacteriologically sterile. Exact observations can thus be made on many phases of the life history.

Earliana tomato is used as the host plant. Seeds are disinfected with calcium hypochlorite (Wilson, 1915), and allowed to germinate in agar plates. The seedlings are transferred to fresh plates of a plant-nutrient medium.[5] One seedling is planted in each plate just before the agar sets, and for most of the cultures one nematode in the larval stage is placed beside the root tip. For the multiple infestations an egg mass was placed near the seedling, which was later found to harbor from 1 to 10 or more developing nematodes. Some larvae have difficulty in penetrating the root, but with good material galls appear in from one to five days, according to the temperature.

The plates can be kept for observation for a week or two, but better growth is obtained if the infested host plant, or part of its root, is transferred to a test tube containing Pfeffer's solution. A few grains of sand are added to each tube for a possible value in aeration.[6] The tubes can be kept sterile for weeks or months, and incubated as desired.

For obtaining uncontaminated larvae, the most practicable source of material is an infested potato. Here the egg masses are protected by a tough brownish case, which appears to be a shell of crushed plant tissue possibly impregnated with the oöthecal secretion discharged by the

[5] An agar medium is made with Pfeffer's solution (Robbins, 1922), modified by the substitution of ferric tartrate for ferric chloride (Hoagland, 1919) and by the addition of boron (Sommer and Lipman, 1926). The following formula was used: $Ca(NO_3)_2 \cdot 4H_2O$, 4.0 grams; $KH_2PO_4$, 1.0 gram; $KNO_3$, 1.0 gram; KCl, 0.5 gram; $MgSO_4 \cdot 7H_2O$, 1.0 gram; ferric tartrate, trace; borax, 1:1,000 solution, 5 cc; glucose, 120 grams; agar agar, 60 grams; distilled water, 6,000 cc.

[6] Method used by G. Thorne.

nematode before egg-laying. The cases can be dissected out unbroken from the tuber. If they are originally free from contamination, a rinse of 3 to 5 minutes in hydrogen peroxide (c.p., full strength, i.e., 3 per cent) will clean them of any external organisms acquired during the manipulation, which need not be too carefully aseptic. From the peroxide, the egg masses are placed directly on agar plates, where any chance contamination can be discovered. Egg masses from tomato galls have also been used. They are considerably less convenient because the protecting cases are more fragile. It is also difficult to clean contaminating soil from the small galls.

The first larvae to emerge in the plates are those which have hatched some time earlier and lain packed, inactive, within the egg case. Later individuals are evidently newly hatched from the eggs. They wander on or through the soft agar and can be handled conveniently with a pointed splinter of bamboo, as used in Thorne's laboratory. A supply of splinters can be sterilized in test tubes in the hot-air oven.

## ABILITY OF LARVAE TO ENTER ROOTS

In view of the abundance of nematode galls in the field, it was most surprising to meet with considerable difficulty in obtaining galls in cultures, even on healthy growing seedlings. A great difference in the ability of nematodes to penetrate plant tissue was observed in different lots of larvae.

In an attempt to analyze this behavior, over 8,000 larvae have been tested. It is seen in table 1 that larvae raised in root cultures are able to enter fresh roots readily, while an initial infestation from field material was obtained less often. In the itemized daily records, infestation by larvae from cultures has been as high as 90 and 100 per cent of the individuals tested. When larvae hatched from a poor root were active enough to use for $F_1$ cultures, they were as successful in entering the new host as were larvae from a better environment. By contrast, some lots of larvae from potato gave no galls, and only a few of the others gave 50 per cent infestation. Larvae from tomato gave 0 to 30 per cent.

There is no indication that external conditions affect the situation. It appears to be in the nature of some lots of larvae to enter the root tip readily and of other lots to find difficulty in penetration. Several possible explanations have been tested and discarded.

The first suggestion was that there might be a condition of dormancy of the nematodes in a dormant potato, or else that the larvae there were somewhat older and weaker than the normal. Egg masses were then taken

fresh from tomato roots growing in pots. There is no reason to assume that dormancy occurs under greenhouse conditions, yet the proportion of galls formed by these larvae was even lower than by those from potato.

This comparison also answers Steiner's (1925) suggestion that a race of nematodes accustomed to potato might resist a change of host. On the contrary, larvae from potato make more galls in tomato seedlings than do those from greenhouse tomato roots (table 1). The larvae from one lot of potatoes[7] in particular showed a high power of infestation. These nematodes had been in potato only two or three generations, however. Their previous host was tomato.

TABLE 1

ROOT PENETRATION BY LARVAE FROM DIFFERENT SOURCES

| Material | Larvae tested | Per cent infestation |
|---|---|---|
| Potato (3 lots, various ages) | 1,810 | 6.0 |
| Tomato (fresh from greenhouse) | 1,599 | 4.0 |
| Potato No. n464 | 91 | 27.5 |
| Tube cultures (mostly from isolated females) | 4,933 | 54.2 |

There is no reason to suspect the peroxide treatment of weakening the eggs or larvae. All the egg masses from No. n464 received this treatment, as did also a few of the galls from cultures. Material so treated gave infestations up to 90 per cent.

Cultured larvae appear exceptionally large and vigorous. The absence of other organisms cannot account for this because the egg masses within an otherwise healthy potato are equally aseptic.

Only two observations seem to have significance: (1) There is an apparent stimulation of larvae which have hatched in a liquid. This has been observed in sterilized Pfeffer's solution, and in tap and distilled water. (2) Larvae hatched and "dormant" within the egg mass in potato, and larvae kept in tubes of any liquid longer than a few weeks, seem to

[7] In December, 1928, potatoes were received from Goodyear's Bar, in the Sierra Nevada foothills. Though stunted by nematode attack, the tubers seemed to have resisted the invaders by walling off many of them with heavy brown tissue. Potatoes grown from this strain failed to carry on the resistance. Small tubers of the third generation were harvested from one can (No. n464) in November, 1929, after four months' growth outdoors in Berkeley. The potatoes were kept in the laboratory all winter, and dissected April 17, 1930. Nematodes were found in only 5 of the 28 tubers, but one of these was packed with nematodes in all stages of development. It was a potato only ¾ inch in diameter. Over a thousand females, large and small, were counted with the naked eye. Three-fourths of the tissue of the tuber showed a watery degeneration from the heavy infestation. The other four tubers had small watery spots packed with nematodes.

be less active in infesting a root. Even cultured larvae become weakened if kept too long in their sterile tubes. Their reserve energy[8] is used up in fruitless activity. In the field, larvae are believed to survive 15 months' starvation (Bessey, 1911). Dryness, temperature, and aeration may have much to do with their survival, and there may in reality be only a small proportion of a population which is able to start the new infestation.

## NUTRITION AND DEVELOPMENT OF NEMATODES IN ROOT CULTURES

Among the seedlings used as host plants, there occurred wide differences in the vigor of root growth. In general, the development of the nematode corresponded to the condition of the host. This statement may seem obvious, yet it becomes significant as the progressive effects of malnutrition are seen, culminating apparently in an alteration of the sex ratio.

In primary cultures, when only one nematode was used for each plant, 50 per cent of the population completed the life cycle from larva to larva ("per cent hatch," table 2), but when one seedling had to support several parasites, only 10 per cent completed the cycle. Again, in single-nematode cultures, only 12 per cent failed to develop as far as sexual differentiation, while in multiple infestations, 61 per cent remained undifferentiated.

A more extreme case of undernourishment occurred in long-unopened cultures ("secondary galls," table 2). Some of the $F_1$ larvae in tubes attacked the root of the host plant from which they had hatched. The plants had been weakened by age and artificial conditions to the point where they no longer provided healthy growing tissue for their parasites. These secondary galls turned brown, and the effect of starvation on the nematodes was conspicuous: 79 per cent remained undifferentiated, and only 2 of the 20 females contained ova, which failed to develop.

Cultural conditions were not always unfavorable for the development of nematodes. In culture No. 2391, one female laid 1,998 eggs, counting 1,406 larvae and 592 unhatched eggs, the highest number of eggs reported for a single female.

Development from larva to larva is possible even in some very unfavorable roots, but the number of eggs laid, and the number, size, and

[8] Godfrey, Oliveira, and Gittel (1933) have demonstrated the food material stored by *Heterodera marioni* as layers of fat in the cells of the intestine.

vigor of larvae are subject to nutritional conditions. Galls can be formed in bits of root tips as short as 5 mm. These conditions are far from normal, but the life cycle has been completed in several such cultures.[9]

From time to time a gall has been found in which the nematode was apparently too weak to lay her eggs, or where external pressure left no room for an egg mass. Larvae inside the body of the female appeared healthy. A similar situation occurs in potatoes, where sometimes the last eggs are not laid by an exhausted female. Nagakura (1930) describes such a female as a *"Brutkapsel";* it may be also the "cyst form" of Jones (1932).

TABLE 2

SEX AND AMOUNT OF DEVELOPMENT OF NEMATODES IN CULTURES

| Type of gall | Infestation | Root growth | Sex undifferentiated* | Males | Females | | | Total number of nematodes | Per cent undifferentiated | Per cent males† | Per cent hatch‡ | Per cent infestation by $F_1$ larvae¶ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | No eggs or no hatch | | Eggs hatched | | | | | |
| | | | | | Insufficient time | Sufficient time | | | | | | |
| Primary | Single | Poor | 63 | 6 | 49 | 94 | 95 | 307 | 20.5 | 2.5 | 37.7 | 58.5 |
| | | Fair | 61 | 2 | 130 | 115 | 158 | 466 | 13.1 | 0.5 | 47.3 | 54.4 |
| | | Good | 39 | 1 | 117 | 158 | 285 | 600 | 6.5 | 0.2 | 59.1 | 53.7 |
| | | All roots | 163 | 9 | 296 | 367 | 538 | 1,373 | 11.9 | 0.7 | 50.4 | 54.2 |
| | Multiple | Poor | 41 | 4 | ..... | 18 | 7 | 70 | 58.6 | 13.8 | 10.6 | ..... |
| | | Fair | 52 | 6 | ..... | 14 | 5 | 77 | 57.5 | 24.0 | 7.0 | ..... |
| | | Good | 3 | 0 | ..... | 4 | 3 | 10 | 30.0 | 0.0 | 30.0 | ..... |
| | | All roots | 96 | 10 | ..... | 36 | 15 | 157 | 61.1 | 16.4 | 10.2 | ..... |
| Secondary | Multiple | Old | 174 | 26 | ..... | 20 | 0 | 220 | 79.1 | 56.5 | 0.0 | ..... |

* These nematodes were allowed sufficient time to complete the life cycle.

† Per cent males is the ratio of males to the total adult population.

‡ Per cent hatch is the percentage of all the nematodes in any series (excluding males, and the females which had insufficient time for development) which completed the life cycle.

¶ Per cent infestation is based on the number of larvae tested. Only a few subcultures were made with $F_1$ larvae from multiple infestations.

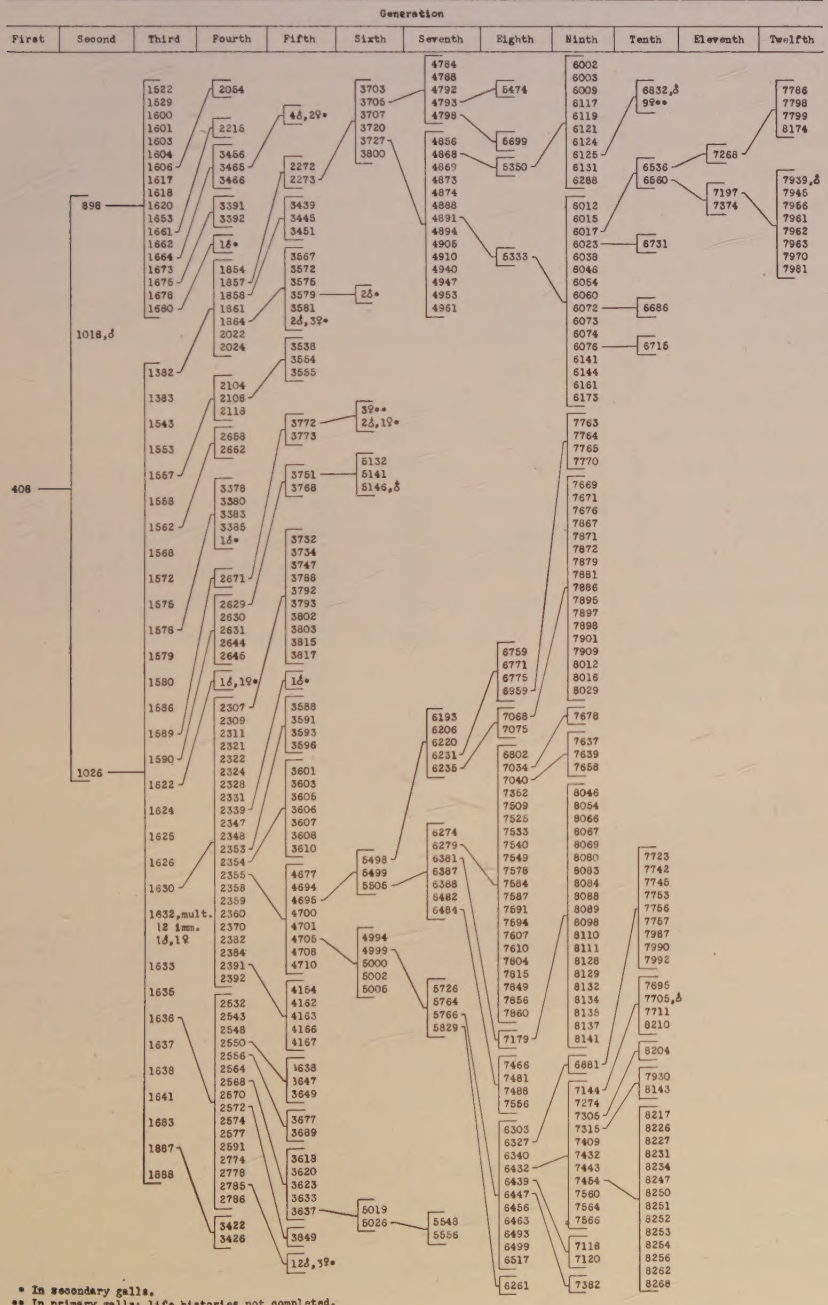
## PEDIGREES

The conditions of the method outlined above are such that, if each plate is planted with only one larva, the fact of reproduction without males can be clearly established. In most cases the unmated females in cultures laid viable eggs. When these eggs hatched, subcultures were started using the aseptic $F_1$ larvae, one to a plate.

[9] Five out of 12 cultures gave the complete cycle. Of the other 7 individuals, 1 remained immature, 2 were males, and 4 were females.

## TABLE 3

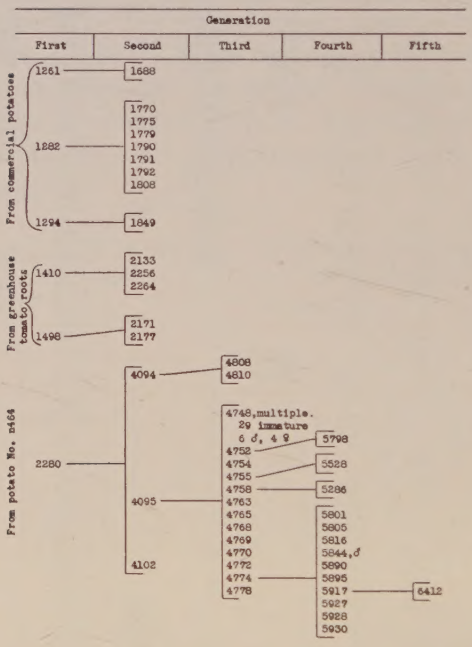
REPRODUCTION BY ISOLATED FEMALES IN ONE FAMILY OF THE ROOT-KNOT NEMATODE




* In secondary galls.
** In primary galls; life histories not completed.

## TABLÆ 4

### Reproduction by Isolated Females in Families from Various Sources




## TABLE 5

### Reproduction and Incidence of Males in First-Generation Cultures of Nematodes from Various Soil Populations

| Source of larvae | | Infestation | Males | Females: sufficient time | | Per cent males |
|---|---|---|---|---|---|---|
| | | | | No eggs or no hatch | Eggs hatched | |
| Commercial potatoes | California, 1929 | Single | 3 | 9 | 17 | 10.3 |
| | | Multiple | 2 | 6 | 2 | 20.0 |
| | Nevada, 1929 | Single | 0 | 4 | 9 | 0.0 |
| | | Multiple | 0 | 11 | 4 | 0.0 |
| | California, 1930 | Single | 0 | 0 | 5 | 0.0 |
| Potato No. n464 | | Single | 0 | 1 | 11 | 0.0 |
| Greenhouse tomato roots | | Single | 0 | 3 | 25 | 0.0 |
| | | Multiple | 1 | 13 | 3 | 5.9 |

One family of the root-knot nematode has been carried through 12 generations by repeated isolations. The pedigree is given in table 3, in which individual nematodes in primary galls are represented by culture numbers. The original parent of the family, No. 408, was a nematode from a commercially grown potato ("California, 1929," in table 5). Each culture number, including No. 408 but excepting those designated as males and one culture designated as multiple, stands for an isolated female with life history complete from larva to larva. Females with incomplete life histories have been omitted from the pedigree, except that in cases where males would otherwise appear to predominate, the number of females with incomplete life histories in primary or in secondary galls is also given, marked with a double or a single asterisk. The occurrence of males in secondary galls is indicated by the number of males, marked with an asterisk.

Additional pedigrees are shown in table 4, one family with 5 complete generations and five with 2 complete generations of isolated females. The 6 nematodes which form the first generation of these 6 families respectively were taken in the egg stage from commercial potatoes ("California, 1929," in table 5), from the home-grown potato, No. n464, and from tomato galls grown in the greenhouse.

Table 5 shows the first generation of nematodes in test-tube cultures started from field or greenhouse populations. It includes the 7 females of the first generation which appear also in tables 3 and 4, making a total of 67 unmated females whose eggs hatched normally, sufficient to show that reproduction without males occurred readily in the first generation of the 5 populations tested. There was a high percentage of males from the first potato population, but because the 5 males were all in poor roots, their occurrence is not inconsistent with the behavior of other material.

## OCCURRENCE OF MALES IN CULTURES

In single-nematode cultures (table 2), there were only 9 males to 1,201 females. There were 10 males to 51 females in the multiple primary infestations, and a still higher ratio of males in the secondary galls, which contained 26 males to only 20 females. Some of the males were less than half the normal length.

The difficulty of development in the more heavily infested roots can be seen by comparing the three types of infestation in table 2. Conditions of nutrition were most favorable in single primary infestations, less so in multiple primary infestations, and conspicuously unfavorable

in the secondary infestations, which were also multiple. Accordingly, the percentage of larvae which were unable to reach the stage of sexual differentiation increased through the series, while the percentage of females whose eggs were able to hatch decreased from single to multiple primary infestations, and in the secondary infestations there were no eggs laid. The increase in percentage of males developed in the cultures is correlated with the increasingly adverse conditions of nutrition.

There is no indication that this is merely a female-producing strain of the root-knot nematode. The occurrence of males appears to follow the same pattern in the different populations used. Table 2 shows 19 males in primary and 26 in secondary infestations. This is the total number of males developed in all cultures. Tables 3, 4, and 5 give their positions in the families, where no genetic relation can be found. The 6 males in original infestations from potato or tomato material (table 5) occurred in poor roots, and unfavorable cultural conditions also prevailed in the secondary galls, which provided by far the highest percentage of males.

The cultures of the sugar-beet nematode made by Berliner and Busch (1914) also produced males in host roots which apparently made little growth. A male developed in a decaying bit of root, in which a second larva failed to grow at all. The male was perfectly formed but abnormally short, because of undernourishment. Another male is described, but only one female, "almost mature."

Although it was not the purpose of this investigation to deal with genetic problems, it does not seem unreasonable to suggest that conditions of nutrition may be important enough in the early stages of development to change the physiological balance of certain individuals. This point of view has been recognized by Babcock and Clausen (1927), who conclude their chapter on sex determination as follows:

> The thesis that sex under normal conditions is determined at the time of fertilization is supported by abundant cytological and genetic investigations. This theory is not inconsistent with the view that the differentiation of sex during development depends upon a complex series of interactions between factors located in the sex chromosomes and autosomes; nor with the observation that internal secretions of the gonads and other glands may play an important part in the process. Nor is it inconsistent with the view that the sexes may be characterized by differences in metabolic rate, nor that changes in the metabolic rate or alterations in the type of internal secretions circulating in the blood during development may go so far as to reverse completely the sex determined by the original zygotic constitution.

It may be interesting to note that Allen (1932) compares hermaphroditism in animals with the situation in monoecious plants, where "the potentialities for the production of the characters of both sexes must

reside in each individual," and the expression of either set of characters may be favored or inhibited by additional factors, either genetic or environmental.

There is a temptation to ask whether fertilization may not be necessary or helpful at the very time the males appear, i.e., when a population has been weakened by malnutrition; but that would be to plead the direct influence of nutrition on sex. Furthermore, it is not known whether these or any of the males are functional, nor whether fertilization may be haphazard. Maupas (1900) considers that the few males which occur in parthenogenetic species are only atavistic, and lack the mating instinct.

Any conclusions must depend, of course, on a study of the germ cells. In *Ascaris* (Edwards, 1910) and in some other nematodes (Gulick, 1911) sex determination is of the XY type. There is no information on the chromosome behavior of *Heterodera*, nor is there cytological evidence on the question of parthenogenesis vs. syngonism.[10]

## OCCURRENCE OF MALES IN ROOTS GROWN IN SOIL

Occasional lots of field material have been found with a preponderance of males. This condition was conspicuous in strawberry roots from San Luis Obispo County. Nearly every gall contained 1 or 2 males, and some showed 5 and 6 males to 1 female. These males seemed unusually short and broad. Strawberry roots obtained from the same field a month later yielded fewer males.

The value of a census taken by dissection of galls and egg masses is limited because of the free traveling of the males. However, when the question was raised, counts were made by dissection of various lots of material. They are given in table 6 as a matter of general interest. There is no correlation between the different percentages, and more cases would be needed before conclusions could be drawn.

The lack of males was not observed in seedlings grown in jars of soil for temperature experiments in 1925 and 1927. The roots were dissected before the males had time to wander, and data are thus more complete than for random field collections. But because the small seedlings were heavily attacked from the beginning, neither the plants nor the

[10] Atkinson (1889) found spermatozoa in the oviducts of the root-knot nematode. Nagakura (1930) describes and figures the seminal receptacles, in which he saw many spermatozoa. He states that dead males were frequently found among the eggs in the oötheca. These reports raise more questions for the cytologist to answer. Von Sengbusch (1927) observed copulation twice in experiments with the sugar-beet nematode, and found spermatozoa in most of the females examined. Considering the abundance of males in the sugar-beet nematode (Molz, 1920), it is very possible that normal mating may occur in that species.

nematodes had a fair chance to develop normally. For completeness of count, the tube cultures are of course the most accurate of all, and it is there that the fewest males are found.

In the 1931 temperature experiments, in soil between 12° and 26° C, only 8 males were found, mostly in decaying galls, while there were 992 females, in roots of every degree of vigor. However, in 3 cages at 28° C, there were 74 males to 354 females. The air bath used in this experiment did not favor a healthy growth of the host plants. The soil dried rapidly.

TABLE 6

MALES FOUND IN ROOTS GROWN IN SOIL

| Host plant | Source | Males | Females | Per cent males |
|---|---|---|---|---|
| Strawberry | San Luis Obispo County | 48 | 29 | 62.3 |
| | San Luis Obispo County, second shipment | 27 | 72 | 27.3 |
| | Carlsbad, California | 1 | 36 | 2.7 |
| | Old roots in formalin | 1 | 25 | 3.8 |
| Potato | From Nevada, 1929 | 0 | 41 | 0.0 |
| | From California, 1930 | 0 | 24 | 0.0 |
| Tomato | Fresh from greenhouse | 19* | 178 | 9.6* |
| | Later generations of strawberry strain from Carlsbad | 6 | 23 | 20.7 |
| | Later generations of strawberry strain, second shipment from San Luis Obispo County | 22 | 329 | 6.3 |
| | Temperature experiments (not at extreme temperatures): 1925 | 15 | 89 | 14.4 |
| | 1927 | 70 | 1,014 | 6.5 |
| | 1931: 28° C | 74 | 354 | 17.3 |
| | 1931: Other cages | 8 | 992 | 0.8 |
| Totals | | 291 | 3,206 | 8.3 |

* Of the 19 males, adult or developing, noted above from tomato, 10 were found in one large gall. he percentage of males is raised by this one gall.

The roots were too heavily infested with nematodes: 46 of the 74 males were developed in crowded galls. Temperature does not directly influence the sex ratio, for in experiments with 835 single-nematode cultures at all temperatures, including the biological extremes, there were only 5 males—all in poor roots. There were no males at all in the culture-solution experiments between 25° and 35°.

On two other occasions a "nest" of males has been found in one gall, an obvious case of overcrowding. One was a tomato root fresh from the greenhouse. Ten males were found in one large gall. In the 1925 temperature experiments, one gall contained 6 males and 2 young females.

There was crowding in tube cultures also. The 14 secondary galls in culture No. 2785 gave a count of 12 males, 3 immature females, and 9 individuals in earlier stages of development. In culture No. 4748 the

primary infestation was too heavy for good growth, and dissection of 6 galls showed 29 individuals in early stages, 6 males, and 4 females without eggs. These two cultures occurred in different families, as can be seen in tables 3 and 4.

Molz (1920) found that in the sugar-beet nematode the development of females was favored by a heavy nitrogen fertilization and by other conditions which promoted a vigorous growth of the host plants, and that a larger proportion of males appeared when the host plants were weakened for any reason. According to his figures, males of that species are usually more numerous than females.

Hornburg (1929) was able to raise the ratio of males of the sugar-beet nematode from 90 to 500 per 100 females, or to reduce it from 300 to 90 per 100 females. His treatment, watering the host plant with infusions of other plants or of seeds, did not affect the amount of infestation.

## SUMMARY

A method of obtaining uncontaminated larvae and of raising them in sterile seedlings is described. This method of cultivation lends itself to the detailed study of a variety of problems.

Larvae raised in root cultures were much more active than larvae from the field in entering growing seedlings in vitro. There was also a great variation in this respect among different lots of nematodes grown in soil, related in part but not wholly to the freshness of the larvae.

A healthy condition of the host plant is important for the development of its parasites.

Reproduction without males appears to be regular and normal for the root-knot nematode. One family has been carried through 12 complete generations by repeated isolations, and the same type of reproduction has been demonstrated in other families from various sources.

The occurrence of males is rare: only 0.7 per cent have been found in single-nematode cultures. Males appeared more frequently in old, unhealthy, or heavily parasitized roots. There were 16.4 per cent in multiple primary infestations, while in secondary infestations, also multiple, 56.5 per cent of the nematodes which were able to develop were males, a ratio of 130 males to 100 females. Observations are presented which suggest that in the field also males occur under adverse conditions.

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# DEVELOPMENT OF THE ROOT-KNOT NEMATODE AS AFFECTED BY TEMPERATURE

JOCELYN TYLER

# DEVELOPMENT OF THE ROOT-KNOT NEMATODE AS AFFECTED BY TEMPERATURE[1]

JOCELYN TYLER[2]

## INTRODUCTION

It is well known that invertebrate animals are dependent on the temperature of the environment for their vital activities. In the case of the root-knot nematode, *Heterodera marioni* (Cornu), this is a factor which must be considered in every phase of biological study. Since it has a direct influence on the rate of metabolism, it is obvious that it must also have an important bearing on problems to which it has not heretofore been applied, such as rate of travel through soil, rate of killing by chemicals, and rate of starvation in fallow fields, as well as on the amount of infestation and the damage done to crops, which have been investigated by Godfrey (1926) and by L. H. Jones (1932).

Temperature is not, however, the only factor influencing the rate of development of this nematode. Godfrey and Oliveira (1932) grew cowpea and pineapple plants side by side in the greenhouse. Yet under identical conditions, development to egg-laying took 35 days in pineapple and only 19 days in cowpea.

Baunacke (1922) has analyzed the effect of temperature on the sugar-beet nematode. His thesis is that the larva, which may normally survive in the soil for months without feeding, depends on a food reserve which it has stored up from the egg. When the soil is cool the larvae are fairly inactive, and the reserve will be used slowly. With higher temperatures, motion and also sense perception are accelerated, so that the larvae should be able to find host plants before the food reserves become exhausted. For the sugar-beet nematode he gives the optimum temperature as 25° C, with a maximum activity at 28°. This increased activity is an escape reaction, which should in nature assist the migration of the larva to a host root or to a cooler environment. If prolonged, it causes death by exhaustion as an indirect result of the high temperature.

In the present paper, it is proposed to analyze the reactions of the root-knot nematode to temperature, as nearly as possible apart from

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other factors. One of the principal objects has been to determine the shortest time for development at different temperatures. The conclusions may not hold good for all hosts, as Godfrey and Oliveira (1932) have demonstrated, but the tomato plant is a very favorable host and the minimum records obtained for it are probably not far from the minimums for most other plants.

## METHODS OF EXPERIMENTATION

Earliana tomato was used exclusively as the host plant. Infested roots were grown at controlled temperatures in three series of experiments, one in Pfeffer's solution and two in soil. Results were consistent throughout the three series.

The aseptic seedling cultures described in the foregoing paper (Tyler, 1933) made suitable material for temperature experiments. In order to study the behavior of individual nematodes, only one worm was used in a culture, and the time was recorded for each culture separately, starting at the first sign of gall formation, when the seedling was transplanted to a test tube containing Pfeffer's solution and placed in an incubator chamber.

In 1927, experiments were made with tomato plants growing in jars of soil, in a water bath patterned after the tanks installed at Johns Hopkins University (Livingston and Fawcett, 1920). The water was kept in continuous circulation around the jars by bubbling compressed air up from the bottom of the tank through a series of holes in a tube the length of each compartment. Battery jars were filled with infested soil from the greenhouse, subirrigated by the method of Jones and Tisdale (1921). When this soil had been brought to the desired temperature, nematode-free seedlings were transplanted into it and the records of the experiment were started. The soil temperature was guarded by an insulation of mineral wool (Jones and Tisdale, 1921). The tops of the plants were in the air of the room.

In 1931 plants were grown in the type of root cages described by Dean (1929), some in cold storage and others in an air bath heated by thermostatically controlled light bulbs, with air circulation supplied by a small electric fan. Records were started when a water suspension of active larvae was poured directly over the previously uninfested roots, where they grew along the glass at the side of the cage.

Standardized thermometers were kept in positions where they would show the actual temperatures of the roots. For soil experiments they were set into the soil at the depth of the galls. For culture-solution experiments they were placed in tubes containing tap water, two for

each basket of culture tubes. Charts of the root temperatures were made from thermograph records checked against the thermometer readings. A fluctuation of ± 1° C was unavoidable in some of the experiments. Any wider fluctuations were only temporary. Four or five accidents occurred in the different experiments, giving a deviation of 3° to 12°, but not reaching extreme temperatures. In most cases, the fluctuations were only a fraction of a degree beyond the limits established. The total time for both types of variation was never more than 20 per cent of the time of any of the experiments reported, and generally much less.

Moisture control was not considered necessary in the soil experiments, because this nematode thrives, especially in the gall, throughout the moisture range of good plant growth (Godfrey, 1926).

Artificial light was supplied for plants growing in soil. Some of the roots in culture solution grew as well with stem and leaves removed as did others with top parts intact.

## METHODS OF MEASURING DEVELOPMENT

The stages of development from stage 3, the free-living larva, to stage 12, the full-grown female, are numbered in accordance with plate 1 of Bessey's paper (1911), which is reproduced in part in figure 1. After the nematode has reached stage 12, the gelatinous oöthecal secretion is extruded from the vulva, and somewhat later small ova can be found in the oviducts. The final stage for these experiments is the extrusion of the first eggs. The metamorphosis of the male is shown in stages 13, 14, and 15.

Each record shows the stage of development reached by one nematode in a certain number of days at a certain temperature. Since the galls were dissected for examination, only one observation could be made on each nematode, and the slower cases are open to several possible interpretations. There are individual differences in rate of development, as will be shown in connection with the rate of egg-laying. In some cases, however, development is undoubtedly arrested by unfavorable conditions, and this may have occurred some time before the examination, so that there is no way of determining when the recorded stage was reached except by comparison with other individuals at the same temperature. Allowance should also be made in soil experiments for delayed infestation. The minimum-time records are more significant because they give definite evidence of what really happened during the time of the experiment.

The time and temperature relations may be expressed in heat units. Each Centigrade degree above 10°, acting for one hour, is counted as

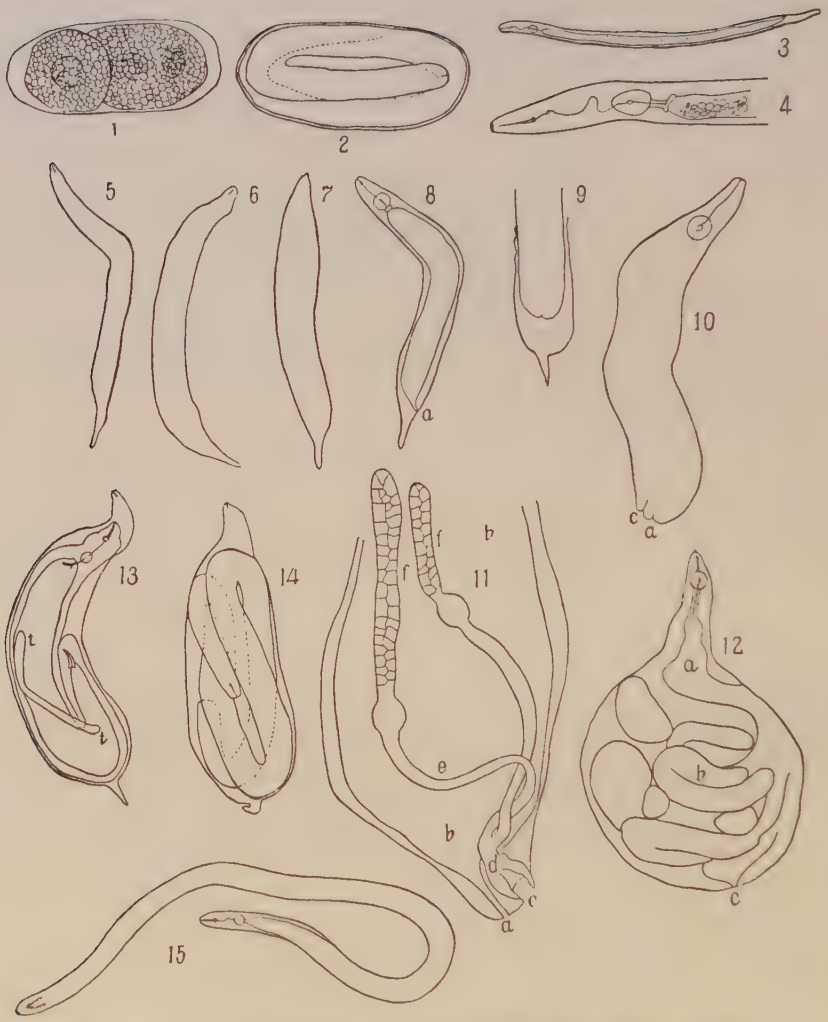


Fig. 1. Stages in the development of the root-knot nematode. [After Bessey (1911), by permission from the United States Department of Agriculture.] 1 and 2, Developing eggs. 3 and 4, Free-living larva and its anterior portion. 5 to 8, Stages in growth of larvae. 9, Posterior portion of immature female just before molting, recognized by the old larval tail which has not been stretched along with the rest of the cuticle. 10, Female after molt: *a*, anus; *c*, vulva. 11, Posterior portion of young female. 12, Full-grown female: *a*, alimentary canal; *b*, loop of oviduct; *c*, vulva. 13 and 14, Metamorphosis of male. 15, Free adult male.

one effective unit. A temperature of 16° for 24 hours is thus counted as $24 \times 6 = 144$ units. Summation of these units gives a measure of the heat requirements, which are approximately constant for a given stage of the life history at any medial temperature, and which increase with succeeding stages. For example, the normal range was as follows, both in soil and in culture-solution experiments: 500 heat units for gall formation by larvae; 2,100–4,000 for development of a nematode from the appearance of a gall to stage 9; 3,700–6,000 to stage 10; 4,800–7,000 to stage 12; 6,300–7,500 to the formation of ova; 6,500–8,000 to the beginning of egg-laying; and roughly 5,000 more for the development and hatching of eggs. Shelford (1927) has pointed out several objections to the summation method. It has nevertheless a practical usefulness, and its inaccuracies are no greater than the variations shown by individual nematodes. It also emphasizes certain differences between mathematical expectations and experimental results, such as the amount of development at very low temperatures, and the different threshold relations of young and mature stages.

For any stage of development, the time and temperature records of individual nematodes at medial temperatures fall roughly into a parabolic curve (fig. 2), practically paralleling the curve of hour-Centigrade-degree units. A heat-unit curve can thus be used as a working basis for predicting the time required for development at any given temperature, within the medial range, and is useful in comparing records obtained at different temperatures.

Heat units were computed empirically from 10° C because 11.5°, the "$a$ point" of the rate curve (fig. 3), was too high for many of the low-temperature experiments, while 9°, which is close to the true threshold temperature for early development at least, is too far from this curve. The threshold of development is below the parabola and the rate curve in any case (Krogh, 1914), and parabolas calculated from 10° are harmonious with the majority of the data from experimentation.

In cultures held at 10° or 12° C for a long period, considerable development may be shown where only a few hundred heat units are recorded. In such cases the threshold temperature is obviously below 10°. At these low temperatures the sum of heat units above 9° is often two or three times the number computed from 10°, although in brief exposures at higher temperatures the difference is negligible. In such special cases, the usual computation gives a false picture of the situation, and the rate of development can be better interpreted by calculating heat units from the threshold, 9°. Where these are mentioned both counts are given, but unless explicitly stated heat units are computed only from 10°.

## TABLE 1

TIME REQUIRED FOR THE DEVELOPMENT OF NEMATODES IN CULTURE-SOLUTION EXPERIMENTS, STARTING FROM APPEARANCE OF GALL

| Temp., °C | To stage 8 | | To stage 9 | | To stage 10 | | To stage 12 | | To extrusion of oötheca | | To oviposition | | | To hatching of eggs | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days | Nematodes | Days | Nematodes | Days | Nematodes | Days | Nematodes | Days | Nematodes | Days | Nematodes | Eggs laid per female | Days | Cultures |
| 31.5 | | | | | 9* | 1 | | | | | 16* | 1 | 19 | | |
| 30.0 | | | 10 | 1 | | | | | | | | | | | |
| 29.5 | | | | | | | 15–17 | 2 | 10*–17 | 2 | 18 –20 | 3 | 7– 75 | 31 –35 | 2 |
| 29.0 | | | 8 | 1 | 15 | 1 | 16 –17 | 6 | 17 | 1 | 17 | 4 | 1– 7 | | |
| 26.0 | | | | | 12 –16 | 2 | | | 17 | 1 | | | | | |
| 25.0 | | | | | | | 19 –20 | 3 | | | 19 –21 | 4 | 2– 50 | 35 | 1 |
| 24.5 | 11 | 1 | 8 –10 | 2 | 19 | 1 | 16 –20 | 5 | 18 –21 | 3 | 19*–25 | 6 | 1– 50 | 29*†–39 | 4 |
| 24.0 | 7 –11 | 2 | 10 –12 | 2 | 11* | 1 | 19 –24 | 19 | 24 | 2 | 22 –26 | 16 | 1–121 | 35 | 1 |
| 23.5 | | | 7* | 1 | 19 | 1 | 21 –25 | 4 | 21 –24 | 3 | 21*–27 | 7 | 8–236 | | |
| 22.5 | | | | | | | 21 | 1 | | | 24* | 1 | 28 | | |
| 22.0 | | | 13 | 1 | | | | | | | | | | | |
| 18.5 | | | | | | | | | | | 42 | 1 | 5 | | |
| 18.0 | | | | | | | 42 | 1 | | | | | | | |
| 17.5 | | | | | | | 34*–45 | 4 | 44 | 2 | 44*–47 | 5 | 1– 20 | 79* | 1 |
| 17.0 | | | | | | | | | | | 50 –58 | 13 | 1– 65 | 83 | 1 |
| 16.5 | | | | | | | 50 –54 | 7 | 52 –53 | 2 | 53*–56 | 4 | 1– 30 | 84* | 1 |
| 16.0 | | | | | | | 56 | 2 | 56 | 1 | 59 –63 | 2 | 1– 40 | | |
| 15.5 | | | | | | | | | | | 64 –68 | 4 | 1– 9 | | |
| 15.0 | | | | | | | 69 –70 | 3 | 68 | 1 | 69*–76 | 6 | 1– 30 | | |
| 14.5 | | | | | | | 68 –73 | 4• | | | 75*–80 | 7 | 1 40 | | |
| 14.0 | | | | | 46* | 1 | | | | | | | | | |
| 13.3 | | | | | 54* | 1 | 103 | 1 | 96–104 | 2 | | | | | |
| 13.0 | | | | | | | | | 91* | 1 | | | | | |
| 12.5 | 49 | 1 | 53 | 2 | | | | | | | | | | | |
| 12.0 | 26* | 1 | 46*–103 | 7 | 69*–110 | 9 | | | | | | | | | |
| 11.5 | 49*–100 | 6 | 82 – 97 | 8 | 82*–110 | 9 | | | | | | | | | |
| 11.0 | 78 – 83 | 2 | 84 | 1 | | | | | | | | | | | |
| 10.7 | 109 –161 | 6 | 110 –157 | 2 | 151* | 1 | | | | | | | | | |
| 10.0 | 92 –162 | 10 | 99 –148 | 2 | | | | | | | | | | | |
| 9.5 | 149 –163 | 3 | 163* | 1 | | | | | | | | | | | |

* Record of minimum time at this temperature plotted in figure 2. In 3 cases, records of egg-laying at two temperatures a quarter of a degree apart, not shown separately in the table, are both included in figure 2.

† In the 29-day culture, plotted in figure 2, larvae were fully developed but not hatched from the eggs.

## RATE OF DEVELOPMENT AT DIFFERENT TEMPERATURES

Tables 1 and 2 present the experimental data in condensed form. A wide range in rate of development is included, but the very slowest cases have been discounted and their records omitted.

From these data, examples of the most rapid rate of development for each stage have been selected for figure 2, a graph which shows the time of development at various temperatures and the relation of the different stages to the parabolas. The culture-solution experiments were started after gall formation, while the soil experiments were started with free-living larvae. In the graph the former records have been given their proper positions in relation to the minimum time required for gall formation, represented by the parabola of 500 heat units, so that the stages of one complete life history can be pieced together from the several observations.

TABLE 2

TIME OF DEVELOPMENT OF NEMATODES IN SOIL EXPERIMENTS, STARTING WITH FREE-LIVING LARVAE

| Temp., °C | To stage 8 | | To stage 9 | | To stage 10 | | To stage 12 | | To oviposition | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days | Nematodes | Days | Nematodes | Days | Nematodes | Days | Nematodes | Days | Nematodes | Eggs laid per female |
| 35.5 | 7*- 8 | 2 | 10 | 1 | | | | | | | |
| 34.0 | 10 | 1 | 12 | 1 | 12* | 1 | | | | | |
| 33.5 | | | 8* | 3 | 14 | 3 | | | | | |
| 31.5 | 10 | 1 | 7*-13 | 3 | 13 | 2 | 14*-17 | 6 | 17*-22 | 5 | 1- 25 |
| 30.0 | 7 | 1 | 10 | 1 | 13 | 3 | 13 -16 | 7 | 17 -18 | 2 | 17- 28 |
| 28.5 | 7 -10 | 11 | 7*-10 | 13 | 12 -14 | 34 | 13 -18 | 53 | 17 | 7 | 1- 50 |
| 28.0 | | | 10 | 1 | 10* | 1 | 13*-17 | 64 | 17 | 3 | 1 |
| 27.5 | 7 -10 | 21 | 7 -10 | 10 | 15 | 3 | 17 | 44 | 17 | 4 | 1- 5 |
| 27.0 | 7 -10 | 9 | 7 -10 | 5 | 12 -15 | 6 | 15 -17 | 59 | 16*-19 | 5 | 1- 15 |
| 26.5 | 8 | 4 | 10 -12 | 2 | 14 | 3 | 17 -19 | 11 | 20 -21 | 38 | 1-150 |
| 26.0 | 6 -10 | 3 | 7*-12 | 4 | 13 -17 | 6 | 19 | 1 | 21 | 75 | 2-150 |
| 25.5 | 11 | 1 | 11 | 1 | 14 -16 | 9 | 16 -20 | 37 | 20*-22 | 26 | 1- 50 |
| 25.0 | 6 -10 | 13 | 8 -13 | 4 | 13 -16 | 4 | 16*-20 | 4 | | | |
| 24.5 | 7 | 1 | 14 | 1 | 17 -21 | 9 | 20 -22 | 58 | 21 -30 | 10 | 3- 80 |
| 24.0 | 6*-12 | 57 | 9 -15 | 14 | 14 -20 | 34 | 18 -20 | 3 | 25 -27 | 25 | 15-150 |
| 23.5 | 9 | 8 | | | 19 -21 | 9 | 19*-24 | 24 | 28 -29 | 9 | 25-100 |
| 23.0 | 9 -13 | 14 | 13 -16 | 4 | | | | | | | |
| 22.5 | 11 -12 | 4 | 11 -16 | 43 | 17 -18 | 14 | 25 -26 | 35 | 26 | 5 | 1- 20 |
| 20.0 | 11 | 1 | 14* | 1 | 25 | 1 | 26*-31 | 2 | 31* | 1 | 15 |
| 19.0 | 17 -20 | 18 | 17*-20 | 13 | 26 -29 | 5 | 38 | 4 | | | |
| 15.5 | | | | | | | | | 67 | 2 | 5- 35 |
| 14.0 | 44 | 1 | | | | | | | | | |
| 13.5 | | | | | 56* | 4 | 73* | 2 | | | |
| 12.8 | 41 | 1 | | | | | | | | | |
| 12.2 | 50 | 2 | | | | | | | | | |

* Record of minimum time at this temperature plotted in figure 2.

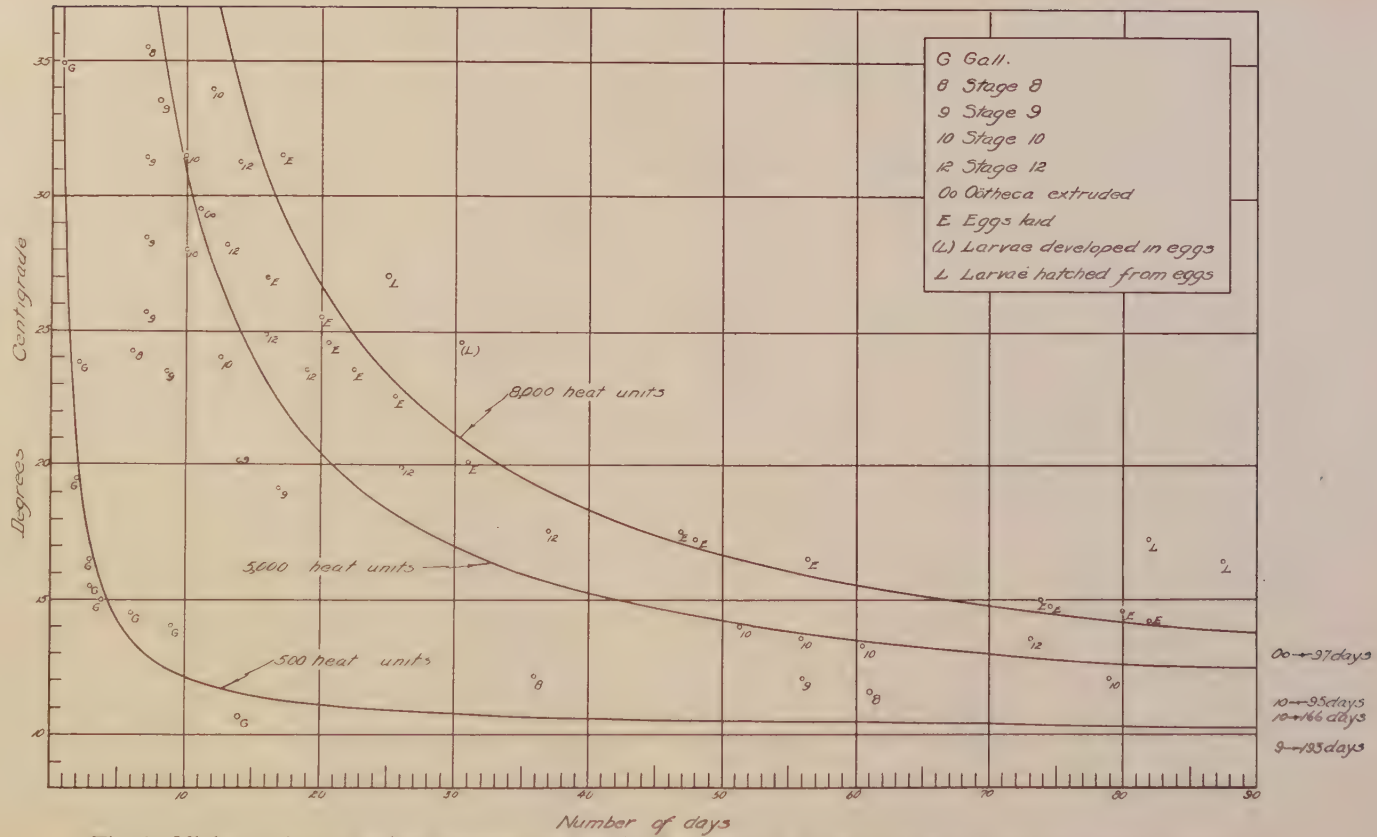


Fig. 2. Minimum time at various temperatures for the development of nematodes from free-living larvae through several stages of growth to the extrusion and hatching of eggs. The curve of 500 heat units above 10° C represents the minimum time allowance for gall formation. The other unit curves are reference lines only.

This graph (fig. 2) shows that the minimum time required for the life cycle in tomato roots from free larva to free larva was 25 days at 27.0° C, increasing to 87 days at 16.5°. The minimum time from the appearance of a gall to the beginning of egg-laying was 15 days at 27.0° and nearly 79 days at 14.3°. In the medial range the rate of increase of time with decreasing temperatures was fairly steady and regular.

TABLE 3

VARIATION IN AMOUNT OF DEVELOPMENT OF NEMATODES UNDER LIKE CONDITIONS

| Temperature, °C | Days | Heat units | Root growth | Number of cultures | Development of nematodes |
|---|---|---|---|---|---|
| 17.0 | 53 | 8,508 | Fair | 1 | Stage 10 |
| | | | | 1 | Female with oötheca |
| | | | | 2 | Females containing full-grown ova |
| 24.0 | 25 | 8,357 | Fair | 1 | Female containing ova |
| | | | | 2 | 6 eggs laid |
| | | | | 1 | 34 eggs laid |
| | | | | 1 | 110 eggs laid |
| | | | | 1 | 121 eggs laid |
| 24.0 | 24 | 8,018 | Good | 1 | Stage 10 |
| | | | | 2 | Stage 12 |
| | | | | 2 | Females with oöthecae |
| | | | | 1 | 20 eggs laid |
| 24.5 | 19 | 6,746 | Good | 1 | Stage 10 |
| | | | | 3 | Females containing ova |
| | | | | 1 | 2 eggs laid |
| 29.0 | 16 | 7,232 | Fair | 1 | Stage 9 |
| | | | | 1 | Stage 10 |
| | | | | 1 | Female containing ova |

The rate of development is dependent to an appreciable extent on the factor of nutrition, which cannot be controlled without a method of completely artificial cultivation. Even at moderate temperatures there was considerable variation in rate or in amount of development in Pfeffer's solution cultures that were identical in all external conditions, including a similar amount of root growth. The data for several groups of cultures compared in this way are shown in table 3. For each group, the galls were formed on the same day by offspring of one unmated female, and were incubated side by side. The records for 24.5° C show that it was possible under the conditions of that experiment for one female to lay 2 eggs and for three others to contain partly developed ova, while a fourth individual had reached only stage 10. The slower individuals must also be considered, of course, and the average rate of development will be presented later, in connection with figure 3. However, the minimum period of development, as shown in figure 2 by the more advanced

individuals at the various temperatures, is of the greatest practical significance because it is experimentally the most accurate, and because of its application in control operations by the fallow-field and trap-crop methods. The differences in rate of development among cultures in the same host species and the differences found by Godfrey and Oliveira (1932) in different hosts may perhaps be interpreted as different manifestations of the fundamental relation between nutrition and rate of development.

## STATISTICAL PRESENTATION OF RATE OF DEVELOPMENT

Figure 3 is a mean-velocity curve based on the data for egg-laying presented in tables 1 and 2. Each line represents a group of records averaged by the method of least squares. Temperature and rate, the reciprocal of the time of development, are the variable factors compared.

Before they were averaged, the figures were corrected in such a way that all records should cover only the time from the appearance of a gall to the first extrusion of eggs. The minimum time required for gall formation (as shown in figure 2 by the curve of 500 heat units) was therefore subtracted from the recorded time of the soil experiments; and where a female with an egg mass was found in any experiment, the recorded time of her life history was corrected by the allowance of 12 units for each egg laid, which will be explained later.

In spite of the irregularities indicated in table 3, a correlation coefficient of 0.97 was obtained for the 279 records from 14.3° to 28.0° C. To determine the "$\alpha$ point" (Shelford, 1927), the curve for these records has been extrapolated to the temperature axis, which it cuts at 11.5°.

At temperatures under 16.5° C, all the records fall below the first curve. Therefore a second curve, with an $\alpha$ value of 10.2°, has been added to the graph, showing the relatively more rapid rate of development at low temperatures. Although the second curve was made from only 10 records, between 14.3° and 14.8°, its direction is almost identical with one made from the 22 records from 14.3° to 16.3°.

The curve for the 22 records above 28° C, averaged separately, shows the reversal of direction which indicates that the average rate is retarded at high temperatures.

The same types of deviation from the straight line, beyond the temperature limits of normal development, have been found by other investigators (Glenn, 1922; Pearis, 1927; Shelford, 1927), following the observations of Krogh (1914). Shelford (1927) uses the term "medial temperatures" in referring to the temperature range within which the data from experimentation coincide with the parabola of time and temperature or with its reciprocal, the straight line of rate and temperature.

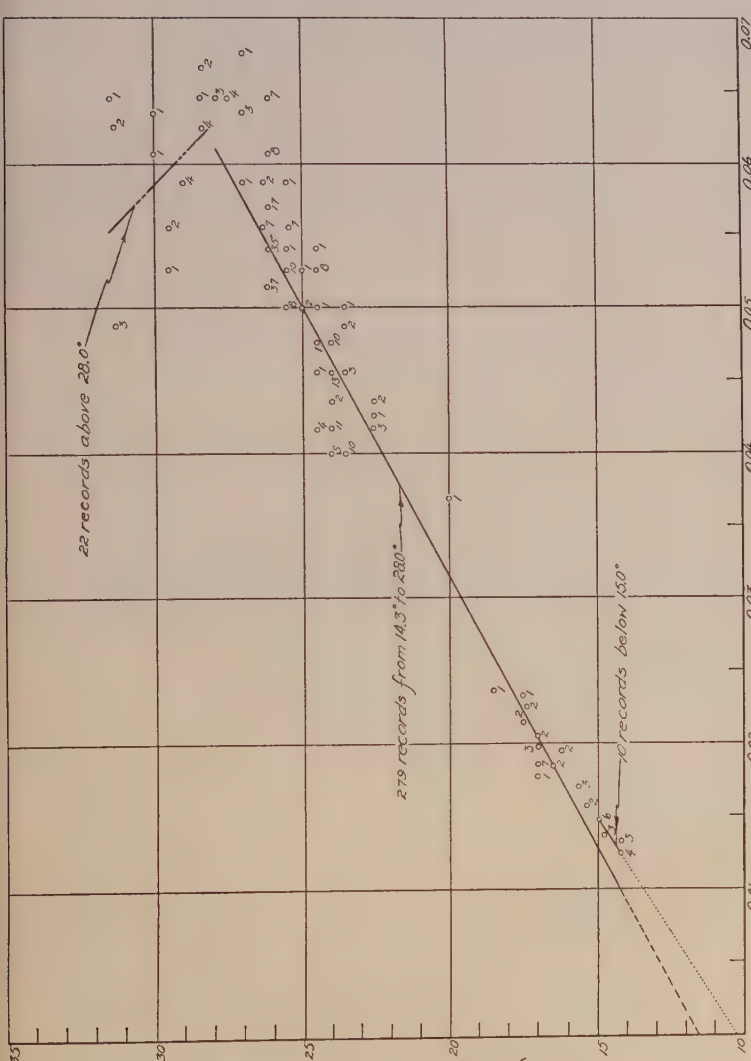


Fig. 3. Rate of development of nematodes from gall formation to egg-laying: an average of 279 records from 14.3° to 28.0° C. This curve has been extrapolated by a dotted line which shows the $\alpha$ point at 11.5° where the line cuts the temperature axis. The 10 records below 15° were averaged again, separately, to form the basis for the lower line with its $\alpha$ point at 10.2°, showing the relatively more rapid rate of development at low temperatures. The 22 records from 28.3° to 31.5° form the upper curve, which shows the rate of retardation at high temperatures. The distribution of the experimental data is indicated by numbers representing nematodes.

## THRESHOLD OF DEVELOPMENT

An experiment was made to test the lowest temperature possible for nematode development, using newly formed galls in Pfeffer's-solution cultures. There was no development in 33 cultures kept between 7.0° and 7.5° C for 50 to 105 days, but in similar cultures stored at this temperature for 58 to 77 days and then removed to room temperature, 5 of the 14 nematodes developed and laid viable eggs.

At 9.5° C there was development as far as stage 9, so the threshold temperature must be at or below 9°. The roots also made some growth.

It is possible that the threshold is higher for maturity and egg formation than for the early stages of development. The velocity curve for the entire life cycle has its $\alpha$ point at 11.5° C (fig. 3), and also the egg-laying records in figure 2 would be more harmonious with parabolas calculated from 11.0°, while stages 10 and 12 are harmonious with the parabolas represented on the graph, which were calculated from 10.0°. Ludwig (1928), working with the Japanese beetle, and Glenn (1922), with the codling moth, found that the egg, larva, and pupa each showed independent threshold and maximum temperatures.

## DEVELOPMENT AT LOW TEMPERATURES

Eggs were laid at 14.3° C, but the greatest development accomplished at a constant lower temperature was the extrusion of the oötheca at 13.3°. This occurred in 3 cultures out of the 13 which had fair root growth and sufficient time allowance. Yet at still lower temperatures, not held constant throughout the experiment, 2 females contained partly developed ova: one culture had been kept for 297 days at several steady temperatures between 7.0° and 12.5°, with a total of 7,722 heat units above 10.0° or 12,642 above 9.0°; the other had been 255 days between 7.0° and 12.5°, plus 9 days at 14.0°, with 6,569 or 10,721 heat units. The failure at 13.3° can probably be explained as the result of inadequate nutrition, but the possible stimulating effects of temperature changes[3] and of subthreshold temperatures (Parker, 1930) should be

[3] Pearis (1927) considers that constant temperatures are unnatural, and may tend to retard development. Ludwig (1928), on the other hand, found that the Japanese beetle developed as rapidly at constant as at fluctuating temperatures, when the effects of extreme temperatures were eliminated and fluctuation took place only between the threshold and the optimum temperature. However, Cook (1927), Pearis (1927) and Parker (1930) have all found an accelerated rate of development for the insects investigated, correlated with daily fluctuations between the threshold and the optimum temperature. The nematode cultures under discussion are not exactly comparable with the experiments quoted from other authors, because the nematodes were exposed to small daily fluctuations of temperature during only a part of the period of development, and for the remaining time the temperature changes occurred at intervals of several weeks.

borne in mind (in connection with the 2 cases where ova were formed at even lower temperatures). However, in the 10 additional cultures at irregular temperatures below 12.5° which had sufficient time and root growth, only one nematode had reached even the oöthecal stage. These cases with irregular temperature records are not included in the tables or graphs, which are based exclusively on experiments at constant temperatures.

At still lower temperatures, maintained more or less constant, there were 334 cultures started at 10°, 11° and 12° C, not all of them reported in the tables. They were examined after several months in cold storage, although their heat-unit counts were still low. Of 320 cultures with less than 4,000 heat units, 90 showed more development than would have been expected from their low unit counts, 95 showed an average rate, and 135 were either very slow or else arrested before examination. Stage 10 was reached 16 times, once with as few as 1,475 heat units above 10°, or 3,400 above 9°, the other 15 times with 2,400 to 3,600 heat units above 10°. The 14 cultures with longer growth time, which had accumulated from 4,000 to 5,200 units above 10°, showed no development beyond stage 10.

Peairs (1927) points out that there is "an apparent loss of vitality incident to the prolonged vital period" at low temperatures. This applies both to host plant and to parasite. Nematodes probably have higher nutritional requirements for maturity and egg formation than for the early stages, while the tomato seedlings failed to continue their growth through the time required for nematode development at these low temperatures. No attempt was made to substitute some species of plant which would make a relatively better growth at low temperatures, because of the host differences found by Godfrey and Oliveira (1932), but such an experiment might give illuminating information.

TABLE 4

Gall Formation in Soil at Low Temperatures

| Temperature, ° C | | Days | Hours above 13° C | Nematodes | |
|---|---|---|---|---|---|
| Range | Average | | | Number | Development |
| 9.0–12.2 | 10.6 | 14* | 0 | 1 | Stage 3 |
| 10.4–13.3 | 12.0 | 43 | 42 | 1 | Stage 7 |
| 10.4–13.7 | 12.2 | 56 | 171 | 2 | Stage 7 |
| 10.4–13.7 | 12.2 | 50 | 171 | 2 | Stage 8 |

* This record is plotted in figure 2.

## ROOT PENETRATION AT LOW TEMPERATURES

Root penetration requires considerable activity on the part of a larva, and its occurrence at very low temperatures was not anticipated. Godfrey (1926) considered 13° C as the critical temperature for gall formation in roots growing in soil. A few galls found at lower temperatures in my 1927 experiments in soil are reported in table 4. In only one case was the soil temperature below 13° during the entire time of the experiment, but the others averaged around 12°. The first record is the only one in soil which shows the time required for gall formation, because the other experiments included a period of larval development within the roots. Experiments on gall formation in petri dishes (discussed in connection with table 10) were not made below 14°.

## DEVELOPMENT AT HIGH TEMPERATURES

While galls were formed and development was started at temperatures as high as 35.0° C, both in soil and in Pfeffer's-solution cultures, complete development was not obtained above 31.5°. In the 22 cases of egg-laying between 28.3° and 31.5°, a few individuals showed the same rate as at 27.0° (fig. 3); but there was no acceleration in response to the higher temperature, and the majority showed a marked retardation.

The curve for rate of development (fig. 3) breaks sharply above 28° C, showing that this is the optimum temperature, above which development is slower. The irregularity of distribution of the records above this temperature is an indication that the limits of normal development have been passed, although the factor of inadequate nutrition again enters at about 31°. Godfrey (1926) states that tomato roots show maximum gall formation at 30°, and that other roots vary somewhat according to the temperature of their optimum growth.

In culture-solution experiments at 36.5° C, the roots made little or no growth and all their growing tips were killed. In 14 to 24 days, 22 nematodes made no growth at all; 12 larvae fed and started to fatten but failed to reach stage 8; and only 1 grew farther, a male, which died before becoming fully developed. This observation is of interest in connection with the discussion of sex ratios in the foregoing paper (Tyler, 1933), but it seems less significant in the temperature study.

After 9 to 16 days at 36.5° C, 7 cultures (table 5) were given time to recover at room temperature. Although the galls were dissected with great care none of the 7 nematodes was found, and it was concluded that

TABLE 5

TESTS OF RECOVERY OF NEMATODES FROM EXPOSURE TO HIGH TEMPERATURES

| Number of cultures | Initial treatment | | | Subsequent incubation | | Total root growth | Nematodes | |
|---|---|---|---|---|---|---|---|---|
| | Temperature, °C | Days | Possible development of nematodes* | Temperature | Days | | Total development | Recovery |
| 1 | 36 5 | 6 | Stage 9 | 25° C | 33 | None | Stage 12 | Partial |
| 1 | | 9 | Stage 10 | 24° C | 30 | Poor | Nematode not found | None |
| 3 | | 14 | Formation of ova | Room | 51 | Poor | Nematodes not found | None |
| 2 | | 15 | Egg-laying | Room | 20 | None | Nematode not found | None |
| | | | | | 51 | None | Nematode not found | None |
| 1 | | 16 | Egg-laying | Room | 51 | Fair | Nematode not found | None |
| 2 | 37.0–39.0† | 4 | Stage 7 | 24° C | 20 | Fair | Stage 3 | None |
| | | | | | | | Extrusion of oötheca | Partial |
| 1 | 37.0–40.0‡ | 1½ | Stage 5 | 31° C | 32 | Fair | Female containing ova | Partial |
| | | | | 25° C | 22 | | | |
| 2 | | 2 | Stage 5 | 31° C | 32 | Fair | Stage 12 | Partial |
| 1 | | 3 | Stage 6 | 31° C | 12 | Fair | Stage 7 | Slight |
| 1 | | 5 | Stage 8 | 31° C | 12 | Poor | Stage 8 | None |
| 2 | | 6 | Stage 9 | 31° C | 12 | Poor | Stage 3 | None |
| 3 | | 8 | Stage 10 | 31° C | 12 | Fair | Stage 3 | None |

* Possible development of nematodes was determined from the data of figure 2, on the basis of time.

† Only 13 hours above 38.0°.

‡ Only 10 hours above 39.0°.

there was no development but probably death in the larval stage. The roots were stunted by the high temperature, and only 2 of them made new growth during the period allowed for recovery at room temperature. In 13 other cultures (table 5) exposed for shorter periods at 36.5° to 39.0°, 5 nematodes made some recovery, but only 1 female formed ova and these were only partially developed.

The records are clear on the subject of recovery, although the nematodes were not examined until the end of the experiment. Their possible development during the time of the initial treatment was tabulated by analogy with the cultures (fig. 2) which showed the greatest development for the given number of days, although as a matter of fact, development is retarded at such a high temperature. If at the end of the experiment a nematode showed more development than was possible during the initial treatment, recovery was recorded. Three of the 5 nematodes showing recovery had suffered only the shortest exposures at the high temperature, and only 1 of the 5 had a theoretically possible development during that treatment beyond stage 8, while the nematodes which made no development at all were those which had suffered the longest exposures to the high temperature.

The irregular results shown in table 5 are to be expected at such an extreme temperature. On the evidence of the 55 complete or partial failures in culture-solution experiments, it is concluded that the maximum temperature for the development of the root-knot nematode in such cultures is around 36.5° C.

Another culture-solution experiment (table 6) showed that an exposure of 5 days at 36.5° C did not kill nematodes which were already in more or less advanced stages of development. Young females continued to form viable eggs when returned to a medial temperature after the exposure. There was some unhealthy development after a 7-days' exposure at 36.5°. Possible development during the initial incubation at 25.0° was calculated by analogy with other cultures at medial temperatures on the basis of heat-unit counts.

It is barely possible that certain resistant individuals might develop at slightly higher temperatures in healthier roots. Indeed, in a preliminary experiment in 1925, 2 females reached stage 12 at temperatures around 40° C. The host plants were growing in jars of infested soil, with their tops in the cooler air of the room. The thermostatic control was accurate, and the other data from this preliminary experiment were similar to those in the later experiments; but because there was no arrangement for circulation of the heated water around the jars, none of the records are tabulated here.

TABLE 6

EFFECTS OF HIGH TEMPERATURE ON PARTIALLY DEVELOPED NEMATODES

| Number of cultures | Initial incubation at 25.0° C | | | | Subsequent treatment | | | | | Nematodes | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | At 36.5° C | | At 25.0° C | | | | |
| | Days | Heat units | Possible development of nematodes* | Root growth | Days | Heat units | Days | Heat units | Additional root growth | Total development | Recovery |
| 1 | 3 | 1,015 | Stage 5 | Healthy | 3 | 1,892 | 37 | 13,381 | Fair | Extrusion of oötheca | Fair |
| 1 | 10 | 3,612 | Stage 9 | Good | 3 | 1,892 | 37 | 13,381 | Good | Female containing ova | Fair |
| 2 | 18 | 6,453 | Extrusion of oötheca | Fair | 3 | 1,892 | 26 | 9,329 | None | Eggs hatched | Good |
| | | | | | | | | | Fair | Stage 12 | None |
| 1 | 3 | 1,055 | Stage 5 | Fair | 5 | 3,171 | 35 | 12,677 | Fair | Eggs hatched | Good |
| 2 | 17 | 6,089 | Stage 12 | Good | 5 | 3,171 | 35 | 12,677 | Fair | Extrusion of oötheca | Poor |
| | | | | Fair | 5 | 3,171 | 35 | 12,677 | Fair | Eggs hatched | Good |
| 1 | 19 | 6,823 | Early egg formation | Good | 5 | 3,171 | 24 | 8,625 | Fair | One egg hatched | Fair |
| 1 | 2 | 721 | Stage 5 | Poor | 7 | 4,553 | 33 | 11,976 | None | Stage 12 | Fair |
| 1 | 10 | 3,612 | Stage 9 | Fair | 7 | 4,553 | 33 | 11,976 | Poor | Stage 14 (male) | Fair |
| 1 | 11 | 3,975 | Stage 9 | Poor | 7 | 4,553 | 33 | 11,976 | None | Female containing ova | Fair |
| 1 | 18 | 6,453 | Extrusion of oötheca | Good | 7 | 4,553 | 33 | 11,976 | None | Stage 12 | None |

* Possible development of nematodes was determined by comparison with other cultures at medial temperatures on the basis of heat-unit counts.

The limit of survival for many animals is around 40° C. Other experiments with nematodes at high temperatures have used only the free-living stages, so that it is not certain whether the high temperature was directly lethal, or whether death was caused by exhaustion of food reserves, as Baunacke (1922) suggested. Frandsen (1916) found that at 40° development of eggs of the root-knot nematode was accelerated for a short time, and that some eggs hatched before the embryos were completely developed, but that after 18 hours at this temperature all eggs and larvae were dead. Hoshino and Godfrey (1933) report that larvae of this species were killed in water at 40° in 2 hours and 15 minutes. Baunacke (1922) found that larvae of the sugar-beet nematode became rapidly exhausted at 29° and above, but that motion continued feebly in some individuals up to 37.5° and even to 39° if heated very gradually. Although all larvae were quiescent at 40°, most of them were able to recover slowly from a short exposure at that temperature.

The fact that the optimum temperature is found so far below the maximum is probably related to the temperature conditions most often occurring in nematode-inhabited soils.

## ROOT PENETRATION AT HIGH TEMPERATURES

Root-penetrating activity of larvae continued at high temperatures, though the results were somewhat irregular. In the 1927 experiments with plants growing in soil, there were 26 roots at 33.0° to 35.0° C without galls, and only 6 roots attacked, in which 8 nematodes were found at stages 5, 6, and 7, and 17 at stages 8, 9, and 10, though they had all had time for further development. There was also a male at stage 14. One gall was found on a root which had been 42 hours in infested soil above 40.5°. From the preliminary experiments of 1925 there are records of 4 galls formed at 40.0°, containing nematodes at stages 8 and 9.

Most of the galls formed in petri-dish cultures at 35° C appeared after 1 or 2 days of incubation (table 10). After this galls were not formed at the high temperature, but some of the larvae survived a 5 days' exposure and were again capable of active root penetration when the plates were removed to a medial temperature. Later development of the nematodes was not affected by the conditions of this experiment.

## COMPUTATION OF RATE OF EGG-LAYING

The rate of egg-laying was reported by Bessey (1911) as 10 to 15 or more eggs a day, without reference to the temperature, and by Byars (1914) as one egg an hour, or one in 50 minutes under the heat of a strong microscope light.

In calculating the rate of egg-laying in my experiments, two methods were used. The first was a trial-and-error method. The number of heat units required for development to the beginning of egg-laying was calculated for 301 females (fig. 3), most of which had laid more than one egg. It was found that an allowance of 12 heat units per egg gave the most consistent results.

The second method, used to check the first, was based on a comparison of pairs of records. If two females cultivated at the same temperature developed at the same rate, they would reach the stage of egg-laying

TABLE 7

VARIATION IN TIME REQUIRED FOR DEVELOPMENT OF NEMATODES FROM GALL FORMATION TO EGG-LAYING IN CULTURE-SOLUTION EXPERIMENTS

| Temperature, °C | Days | Heat units | Number of cultures | Eggs laid |
|---|---|---|---|---|
| 25.0 | 20 | 7,017 | 1* | 2 |
| 24.5 | 19 | 6,562 | 1† | 5 |
| 24.5 | 19 | 6,746 | 1 | 2 |
| 24.0 | 24 | 8,018 | 2 | 7 |
| 24.0 | 25 | 8,357 | 2 | 6‡ |
| 24.0 | 25 | 8,125 | 1 | 8 |
| 23.5 | 21 | 6,698 | 2 | 8 |

* Culture B of table 8.

Culture A of table 8.

Under identical conditions, other nematodes laid from 34 to 121 eggs (see table 3).

with the same number of heat units. On this assumption, if one culture is examined at the beginning of egg-laying and the other is allowed a longer period of development, the difference in number of heat units between the two cultures would correspond to the difference between the two egg counts, and indicate the heat requirements for laying that number of eggs.

However, the rate of development is not the same for all females. Table 3 shows the wide variation in amount of development for the same time and temperature, and table 7 shows the range of time, measured in heat units, required by different nematodes at approximately the same temperature for the same amount of development. Table 3 includes some of the records of very slow development which were omitted from table 1.

Since it would obviously be inaccurate to compare two females for rate of egg-laying unless their rates of development were practically the same for the entire life history, it is necessary to interpret the differences. Simple inspection of the data in tables 1 and 2 shows which cul-

tures made rapid and which slow development. Of the 10 records given in table 7, the culture showing the shortest time to egg-laying is called A, and a somewhat slower culture, B, was selected as having a rate more representative of the group. The 13 "longer-time" cultures at the same temperature, which had from 15 to 390 eggs apiece, were compared with A and with B, as illustrated for 2 of them in table 8. The first of these, No. 4910, averaged 10.7 units per egg when compared with A, and 9.4

TABLE 8

METHOD OF CALCULATING THE NUMBER OF HEAT UNITS FOR EGG-LAYING

| | Comparison with short-time culture A | | Comparison with short-time culture B | |
|---|---|---|---|---|
| | Eggs laid | Heat units | Eggs laid | Heat units |
| Longer-time culture No. 4910 | 390 | 10,687 | 390 | 10,687 |
| Short-time culture | 5 | 6,562 | 2 | 7,017 |
| Difference | 385 | 4,125 | 388 | 3,670 |
| Heat units per egg | ..... | 10.7 | ..... | 9.4 |
| Longer-time culture No. 5798 | 15 | 7,178 | 15 | 7,178 |
| Short-time culture | 5 | 6,562 | 2 | 7,017 |
| Difference | 10 | 616 | 13 | 161 |
| Heat units per egg | .... | 61.6 | .... | 12 4 |

when compared with B, indicating that the entire rate of development of this culture was more rapid than that of B, and even perhaps of A. In the second comparison shown in table 8, culture No. 5798 approached the rate of B, as shown by the average of 12.4 heat units per egg, as against 61.6 units when compared with A. The small number of eggs makes the difference in average number of units per egg more striking, but the retardation indicated by this high average probably occurred throughout development, and should not be referred entirely to the period of egg laying.

Similar comparisons were made with pairs of cultures at 29° C. In most cases at both temperatures, one of the comparisons gave a result between 10 and 20 units per egg, though there were two instances of development so slow that both comparisons gave absurdly high results. It was concluded from this calculation also that 12 units per egg is a reasonable allowance. This would be 1 egg an hour at 22°, or 12 eggs a day at 16°.

## RATE OF DEVELOPMENT OF EGGS

To give information on the complete cycle, the rate of embryonic development was investigated. Recently deposited egg masses from culture-solution experiments were returned to the incubators and watched for larvae. Another and more exact method was to observe the development of individual eggs in distilled water or saline solutions, sealed in hanging-drop cultures. Table 9 reports both of these experiments. There is individual variation, as in all biological material, and the data are less

TABLE 9

TIME REQUIRED FOR DEVELOPMENT AND HATCHING OF EGGS IN VITRO

| Temperature, ° C | Days | Eggs |
|---|---|---|
| 29.5 | 9–11 | 16 |
| 27.0 | 9*–11 | 22 |
| 26.5 | 12–13 | 21 |
| 25.5 | 12–15 | 14 |
| 24.5 | 15–17 | 10 |
| Room | 19–25 | 9 |
| 17.5 | 26 | 1 |
| 17.0 | 28–31 | 17 |
| 16.5 | 31* | 7 |

* Record of minimum time at this temperature plotted in figure 2.

extensive than those on other stages of the life cycle, but they give a consistent curve (fig. 2). Eggs hatched after 9 days at 27.0 C and after 31 days at 16.5°, counting from the 1 or 2-celled stage.

Godfrey and Oliveira (1932) report hatching of eggs in 5 days, at a temperature around the optimum, in galls on cowpea. This is a much shorter time than is found in any of my records, but since these authors allow 19 days before the discovery of the egg mass, the length of the entire cycle, 24 days in their experiment, is only one day less than my most rapid case.

## RATE OF ROOT PENETRATION

The length of time required for gall formation at different temperatures is shown in table 10 and figure 2. As soon as plate cultures were started with one active larva beside a seedling in a petri dish (for method see Tyler, 1933), the plates were placed in an incubator chamber and examined for galls at 12-hour intervals. Galls were formed in plates at

15° C in a minimum of 4 days. The rate increased at higher temperatures, and at 35° macroscopic galls were found within 21 hours. That actual entrance into the root probably occurred sooner than this is indicated by the stained preparations of Godfrey and Oliveira (1932), which demonstrate larvae inside the root tips of cowpea and pineapple

TABLE 10

TIME REQUIRED FOR GALL FORMATION IN TOMATO SEEDLINGS BY NEMATODE LARVAE IN PETRI-DISH EXPERIMENTS*

| Temperature, °C | Days | Number of larvae penetrating roots |
|---|---|---|
| 35.0 | 0.8†–1.0 | 5 |
| | 1.5 –2.5 | 22 |
| | 3.5 | 1 |
| 23.8 | 2.0†–7.0 | 8 |
| 19.5 | 2.0† | 1 |
| | 3.0 –6.0 | 12 |
| 16.5 | 3.0†–4.0 | 7 |
| | 5.0 –11.0 | 9 |
| 16.0 | 6.0 –7.0 | 2 |
| 15.5 | 3.0† | 1 |
| 15.0 | 4.0†– 6.0 | 6 |
| | 11.0 –14.0 | 3 |
| 14.5 | 6.0† | 1 |
| 14.0 | 9.0†–11.0 | 2 |

* There is also a record of gall formation in soil in 42 hours or less at 40.5° to 44.0° C, and another in 14 days at 9.0° to 12.2°.

† Record of minimum time at this temperature plotted in figure 2.

6 hours after inoculation, although galls did not appear until nearly 2 days had elapsed. The discrepancy between the time of root penetration and the appearance of the gall was not so great in my plate cultures. The root tip became opaque while the nematode was still only partly buried in its tissue, and a definite swelling appeared a few hours later.

## SUMMARY

The minimum time required for the life cycle of the root-knot nematode from larva to larva in experiments in tomato roots was 25 days at 27.0° C, increasing to 87 days at 16.5°. Development from gall formation to egg-laying required 15 days at 27.0° and 79 days at 14.3°.

The velocity curve shows acceleration of rate of development with rising temperatures through the medial range, and marked retardation of the average rate above 28.0° C, although cases were found which showed practically the same rate at 31.5° as at 27.0°. Below 16.5° development was relatively more rapid than the average shown by the velocity curve. There are also considerable variations in rate of development for individual nematodes, possibly related to their nutrition.

As a working guide for all stages, heat units were computed as hour-degrees above 10° C. Development to egg-laying required from 6,500 to 8,000 such units.

Root penetration by larvae in soil occurred at temperatures as low as 12.0° C. Early development occurred as low as 9.5° in cultures, indicating that the threshold of development is around 9°. After newly formed galls had been stored for 77 days at a temperature of 7.0°, a few of the nematodes were still able to develop normally at room temperature. No eggs were laid below 14.3°, but whether this limitation was related to temperature or to nutrition was not determined. The threshold temperature for maturity and egg-laying appears to be higher than for the early stages of the life cycle.

Serious injury to developing individuals in cultures was found at 36.5° C, possibly correlated with the condition of the host plant. Free larvae, however, were still capable of root penetration after 5 days in plates at 35.0°, and one gall was found in soil above 40.5°. Eggs were not laid above 31.5°.

The rate of egg-laying was computed roughly as one egg per hour at 22.0° C, or 12 units above 10.0° for each egg laid.

Eggs developed from the 1 or 2-celled stage to hatching in 9 days at 27.0° C and in 31 days at 16.5°.

Root penetration and gall formation in petri-dish cultures required 4 days or more at 15.0° C, decreasing to 21 hours at 35.0°.

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# A COMPARISON OF AONIDIELLA AURANTII AND AONIDIELLA CITRINA, INCLUDING A STUDY OF THE INTERNAL ANATOMY OF THE LATTER[1, 2, 3]

ROBERT G. NEL[4]

This investigation was undertaken for two reasons: first, to determine the actual status of *Aonidiella aurantii* (Mask.) and its so-called variety, *A. citrina* (Coq.); and second, to learn more about the internal anatomy of the Diaspidinae, as certain structural features still seem to be matters of controversy.

Because of their distinct differences in color and mode of attack, *aurantii* and *citrina* are respectively recognized as the red scale and yellow scale. In fact, so specific are they in these differences that their identity is hardly ever confused; to workers in the field the names red and yellow scales mean two different insects. From a systematic viewpoint, however, distinction has not been so clear-cut, as no morphological differences could be detected upon which a differentiation could be based, with the result that *citrina,* instead of being given specific standing, has been classified as a variety of *aurantii.*

## HISTORY AND SYNONYMY

By referring to Fernald's[(8)] *A Catalogue of the Coccidae of the World,* and to subsequent publications on the Coccidae, one finds that *Aonidiella aurantii* (Mask.) has been described under many different names. As a result, a long list of synonyms has gradually been built up. It is not within the scope of this paper to enter into an extended discussion of these synonyms. The writer will, therefore, limit himself only to those main genera that played the most important roles in the complicated systematic history of the species concerned.

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[2] Paper No. 238, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[3] Study begun and carried on for a year at the Citrus Experiment Station, Riverside, and completed at Cornell University as a thesis presented to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1930.

[4] Graduate student, 1929–30, in the Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside.

*Aonidiella aurantii* (Mask.) belongs to the subfamily Diaspidinae, which is generally regarded as probably the best known of the Coccidae. This species was first described by Maskell[22] in 1878, under the genus *Aspidiotus,* and soon after by Comstock,[5] as *Aspidiotus citri.* The latter author, however, upon exchanging specimens with Maskell, immediately came to the conclusion that the two were identical. In 1895, Berlese and Leonardi[1] erected a new genus *Aonidiella,* taking *aurantii* as its type. In 1899, in the supplement of his *Check-List of the Coccidae,* Cockerell[4] placed *aurantii* under *Chrysomphalus,* giving no reference for this change. Since then, especially in the field of economic entomology, workers throughout the world have given *Chrysomphalus* preference as the genus for *aurantii.* However, the Italian school has consistently used *Aonidiella.* In 1921, MacGillivray,[20] in his classification of the Coccidae, also listed *aurantii* under *Aonidiella,* which he recognized as a valid genus.

Specimens of *Aspidiotus hederae* (Vallot) and *Chrysomphalus aonidum* (Linn.), being type species of respective genera, were carefully studied by the writer in order to obtain a better understanding of the generic characters in question. The original and subsequent descriptions were also taken into consideration.

As a result of these studies, the writer feels convinced that not only is *Aonidiella* a valid genus, but that *aurantii* is more properly classified here than under either *Aspidiotus* or *Chrysomphalus.* In fact, the types of *Aspidiotus* and *Chrysomphalus* show a closer relationship to each other than does *aurantii* to *aonidum.* For example, in both *Aspidiotus hederae* and *Chrysomphalus aonidum,* circumgenital glands are present, while both possess bodies generally turbinate in form; circumgenital glands, on the other hand, are wanting in *Aonidiella aurantii,* while its body is typically reniform in shape, as a result of the posterior elongation of the lateral thoracic lobes.

However, in order to determine the exact position of these species and the validity of the above-named genera, a detailed study of the Diaspine group should be undertaken. Time did not permit such an investigation in conjunction with the work described in this paper, as it would take at least a few years to obtain the necessary types.

The first description of *Aonidiella citrina* (Coq.) was made by Coquillett[6] in 1891, who was conducting some spray experiments on scale insects in the San Gabriel Valley, California. He referred to it as follows:

"The orange tree experimented upon was infested with the yellow scale (*Aspidiotus citrinus*)."

Notwithstanding its briefness and inadequacy, this description is recorded as the original.

It seems, however, that Coquillett did describe it more fully as a distinct species, although this description was never published, for the reasons brought out in the following statement, made by Dr. L. O. Howard[13] in 1894:

> The Yellow Scale is mentioned in some California publications as *Aspidiotus citrinus* Coq. and Mr. Craw[5] is of the opinion that it is a distinct species and was imported independently from Japan in 1872 into the San Gabriel Valley. The name *Aspidiotus citrinus* Coq., was sent Professor Riley with a MS description, but from his own careful study in California, and correspondence with Mr. Coquillett, Professor Riley concluded that the structural differences between the two forms are not constant and that *citrinus* can only be considered as a variety.

From then onward, it continued to be classified as a variety of *aurantii,* and thus underwent the same generic changes, i.e., from *Aspidiotus* to *Chrysomphalus* and to *Aonidiella.*

In the following pages the writer has presented the result of a detailed study conducted on both these insects, and has come to the conclusion that there are sufficient morphological as well as biological differences to warrant giving *citrina* specific standing.

## DISTRIBUTION AND ECOLOGY

The red scale is widely known in practically all the subtropical and tropical countries of the world, having been recorded from southern Europe, Syria, Greece, Turkey, Italy, Spain, India, Ceylon, Mauritius, Australia, New Zealand, China, Japan, South Africa, Rhodesia, southern and western United States, Samoa, Java, and the West Indies. The yellow scale is not so widely distributed, having been recorded only from Japan, California, and Texas. It is probable that as a result of its similarity to the red scale, it may have been mistaken for, and recorded as, the latter.

The red scale in California, as noted by Quayle,[28] is chiefly a citrus pest, so that its distribution is governed largely by that host plant. In the citrus area south of the Tehachapi Mountains, this scale occurs in the following counties: Santa Barbara, Ventura, Orange, Los Angeles, Riverside, San Bernardino, and San Diego. In conjunction with the distribution of the yellow scale the above author states:

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[5] Craw, Alexander. Beneficial insects. Fourth Bien. Rept. Calif. Bd. Hort. p. 97. 1894.

It is widely distributed over the citrus belt of southern California, often associated more or less with *aurantii*. In addition to its occurrence in the southern part of the State, it is also found on the citrus trees of the Sacramento Valley where it is the most important scale occurring on citrus trees. In the same section the typical *aurantii* is not known. In the citrus belt of the south the yellow scale occurs in various degrees of severity ranging from occasional scales scattered about on parts of the tree, to badly infested trees.

From its general distribution, the yellow scale apparently shows a preference for the more arid and warmer interior valleys of central California or better withstands lower temperatures, while the red occurs more abundantly in the southern citrus area. In the Redlands-Riverside section, the yellow scale is most abundant in those sheltered groves lying along the foothills.

Perhaps the most important differences between the red and yellow scales are their modes of attack on the host plant. The red scale is found on all parts of the tree, whereas the yellow is limited almost entirely to the leaves and the fruit, a fact that makes the latter a less serious pest.

## LIFE-HISTORY OBSERVATIONS

The data presented here were obtained from records of insects reared on young seedling and Valencia orange trees. The experiments were conducted in the lath house during the warmer months, and were transferred to the insectary with the approach of winter. Table 1 gives the mean minimum and the mean maximum temperatures for the period during which most of the life-history studies were made.

TABLE 1

TEMPERATURES DURING PERIOD OF LIFE-HISTORY WORK

| Year | Month | Mean maximum, degrees Fahrenheit | Mean minimum, degrees Fahrenheit |
|---|---|---|---|
| 1928 | July | 98.64 | 58.80 |
| | August | 95.09 | 58.35 |
| | September | 96.66 | 51.20 |
| | October | 82.48 | 43.84 |
| | November | 74.28 | 39.83 |
| | December | 66.16 | 35.13 |
| 1929 | January | 63.10 | 34.06 |
| | February | 62.90 | 33.52 |
| | March | 70.83 | 39.16 |
| | April | 69.83 | 43.67 |

During the course of these studies, the observations on the habits and the metamorphosis of both the red and the yellow scales were always made on a comparative basis. These observations are fully recorded (tables 2 and 3) and the conditions were identical for both, unless otherwise indicated.

TABLE 2

SUMMARIZED LIFE HISTORY OF AONIDIELLA AURANTII

| Experiment No. | Free-moving larvae | | | Duration molting period, days | | Duration of instars, days | | | Total time, in days, from free-moving stage to start of reproduction | Adults secured | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Date transferred (1928) | Number transferred | Number settled in 24 hours | First | Second | First stage | Second stage | Third stage* | | Male | Female |
| 100 | 8/10 | 55 | 24 | 4 | 3 | 12 | 9 | 32 | 60 | 11 | 16 |
| 105 | 8/31 | 78 | 54 | 3 | 4 | 13 | 9 | 32 | 61 | 14 | 30 |
| 108 | 9/1 | 31 | 28 | 3 | 3 | 15 | 7 | 33 | 62 | 11 | 12 |
| 109 | 9/4 | 30 | 24 | 3 | 4 | 14 | 8 | 31 | 60 | 5 | 13 |
| 110 | 9/5 | 40 | 40 | 4 | 3 | 13 | 8 | 34 | 62 | 9 | 21 |
| 111 | 9/6 | 147 | 128 | 4 | 4 | 13 | 9 | 35 | 63 | 31 | 80 |
| 131 | 9/27 | 160 | 151 | 3 | 4 | 12 | 10 | 31 | 62 | 55 | 77 |
| 132 | 9/29 | 180 | 144 | 3 | 4 | 12 | 9 | 32 | 61 | 66 | 70 |
| Average | | | | 3.38 | 3.63 | 13.00 | 8.63 | 32.50 | 60.26 | | |

* From second molt to beginning of reproduction.

TABLE 3

SUMMARIZED LIFE HISTORY OF AONIDIELLA CITRINA

| Experiment No. | Free-moving larvae | | | Duration molting period, days | | Duration of instars, days | | | Total time, in days, from free-moving stage to start of reproduction | Adults secured | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Date transferred (1928) | Number transferred | Number settled in 24 hours | First | Second | First stage | Second stage | Third stage* | | Male | Female |
| 105 | 8/31 | 60 | 54 | 3 | 2 | 11 | 8 | 38 | 64 | 19 | 14 |
| 112 | 8/14 | 70 | 53 | 3 | 4 | 13 | 11 | 35 | 66 | 30 | 18 |
| 114 | 8/23 | 22 | 17 | 4 | 3 | 14 | 9 | 36 | 66 | 8 | 5 |
| 115 | 8/23 | 16 | 11 | 3 | 5 | 15 | 8 | 35 | 66 | 3 | 3 |
| 117 | 9/1 | 27 | 19 | 2 | 4 | 14 | 9 | 34 | 63 | 14 | 3 |
| 119 | 9/5 | 16 | 13 | 4 | 5 | 13 | 9 | 37 | 68 | 6 | 6 |
| 121 | 9/26 | 48 | 26 | 3 | 3 | 13 | 11 | 36 | 65 | 10 | 15 |
| 123 | 8/27 | 137 | 119 | 3 | 4 | 12 | 8 | 34 | 61 | 51 | 44 |
| Average | | | | 3.13 | 3.75 | 13.13 | 9.13 | 35.63 | 64.88 | | |

* From second molt to beginning of reproduction.

First-stage or free-moving larvae, generally known as "crawlers," were procured by placing heavily infested oranges or lemons in battery jars provided with cheesecloth tops. In order to hasten the production of the larvae, the jars were kept in a fairly warm place. After a few days, the females produced many young which were removed from time to time. With the aid of a field binocular microscope and a fine camel's hair brush, the crawlers were transferred one by one to the leaves of young potted trees. Only those larvae that moved away from where they were placed, were recorded and used for further observation. This precaution was taken for two reasons: In many cases it was noted that when a larva was so transferred as to be accidentally placed on its back, it was unable to right itself, and died. The possibility existed that when an inactive larva was transferred, it was already in the process of settling and had probably pierced the epidermis of the fruit. Such a larva seemed unable to repeat the process of piercing the leaf.

In order to facilitate observation and to prevent the larvae from settling all over the tree, the following measures were adopted: All leaves on which larvae were to be placed were wiped off so as to remove dust, excessive moisture, and gum exudations. The larvae were kept in restricted areas by the use of leaf cages. Each leaf cage consisted of an upper and a lower cardboard frame, both lined on the surfaces adjacent to that of the leaf, with black velvet. This prevented the escape of the larvae under the frame and guarded the leaf against chafing. The upper frame was covered with a celluloid "window," and the lower one left open so that the transpiration of the leaf would not be disturbed. After transferring the larvae to the upper surface of the leaf, the latter was enclosed between the two cardboard frames which were held in position by means of paper clips. Observations could then be made through the celluloid window. The other method consisted of the isolation of the individual leaves. Prior to the transference of the larvae, the leaves were isolated by placing narrow bands of paraffin wax coated with tree tanglefoot, around their petioles. The few larvae which were trapped by the tanglefoot were counted and deducted from the total number transferred.

Of the above methods, the latter is preferable since direct observation under the microscope can be made without disturbing the settled colony. When the leaf cages are used, the larvae tend to crawl between the leaf surface and the velvet, thus becoming completely hidden. Also, the leaves frequently drop off because of the weight of the cages.

*First Larval Stage.*—This stage will be discussed under two subheadings: the active or free-moving stage, and the fixed or settled stage.

The females of both the red and the yellow scales are viviparous. After birth the larvae remain a day or two under the protection afforded by the covering of the mother scale. On lifting a mature female during the reproductive period, a few larvae will always be found clustered close together in the small space around the pygidium. In this space, along with the larvae, the dry discarded amnion coverings are often found.

By treating some of these larvae with a 10 per cent KOH solution and then staining them with acid fuchsin, their mouth parts will show interesting degrees of internal transformation. In individuals recently born, the two pairs of bristlelike mandibles and maxillae are rolled up in two coils like the hairspring of a watch, with one on each side of the brain. This position of the mouth parts is also noticeable during the time that the larvae are still enclosed in their amnion coverings within the oviduct of the mother. The older larvae, which have just made their appearance and are still crawling about, have uncoiled their mouth parts in the form of long loops, each of which is contained within an internal sac-like structure, known as the crumena.

Soon after making their appearance, the young larvae crawl actively about and come to rest when a suitable feeding place has been selected. The preferred place for settling is beside the midrib or some other prominent vein of the leaf. The duration of this active crawling stage is variable, depending largely upon how soon a favorable feeding spot is located. The majority of the larvae have been noticed settling within the first 6 hours, but a few have been found crawling about, 24 hours after liberation.

Mortality was highest during this stage. In the writer's opinion the cause of this lies in the inability of the larvae to pierce the epidermis, resulting in death from starvation; the inability of the larvae to form a protective wax covering, thereby leaving themselves exposed to drying; and dropping off the leaf, resulting in inability to reach the host plant again.

After finding a suitable feeding place, the larva proceeds to pierce the epidermis with its mouth parts. The rostrum is closely pressed against the surface of the leaf so as to support and direct the rostralis, composed of the united mandibles and maxillae, into the tissues. The distal or free end of the rostralis gradually passes through the rostrum, causing the long loop in the crumena to grow smaller and smaller. The piercing is aided by a slowly rotating movement accompanied now and then by a rise and fall of the anterior portion of the body.

Very shortly after the insertion of the mouth parts, the whole dorsal surface of the larva becomes gradually covered with very fine, silvery white, wax threads. As a result of the rotating of the body, these wax threads coalesce and assume the form of a flattened cone or cicatrix which soon covers the whole body. The legs and the antennae are withdrawn beneath the body and the latter becomes more rounded in outline during the formation of the wax covering. Following this and up to the beginning of the first molt, the only noticeable changes are the gradual expansion of the insect beneath the wax covering and a compacting and darkening of the latter around the outside rim. During this whole period the insect remains unattached to its wax covering, which, when it is removed, is soon replaced by the secretion of a new one. Quayle[28] found that:

"In the case of some the covering was removed and the maximum number of new coverings formed was four. When the covering was removed three or four times, the insect usually died."

The first instar ends with the first molt. The duration of this stage was found to be about the same for both the red and the yellow scales, which had an average of 13 and 13.13 days respectively (see tables 2 and 3). A great variation in the degree of development of the individuals in the different lots was noted, evidently due to the fact that in some cases conditions were more favorable for rapid development. There was differentiation of males and females during this stage.

*First Molt.*—Externally the first molt can be recognized by the outer rim or margin of the wax covering, which becomes clear-cut and more compact. If the insect is lifted during this period, it is found firmly attached to its covering, and the body itself is in a turgid condition. The ventral side of the insect, proximad to the leaf surface, is light brown in color and polished in appearance. A microscopic examination of the internal structures during this period, or just prior to the actual molt, shows the developing second stage lying within the body wall of the first stage. Again the coiled mandibles and maxillae are evident on each side of the mouth region. There is, therefore, a short period during the actual molting process and just before the insertion of the new second stage mouth parts, when no food is taken from the plant tissue.

The splitting of the larval skin usually starts in the region of the mouth and then follows along the sides. The dorsal skin is incorporated into the wax covering to which it adheres firmly; the ventral skin which carries the mouth parts, the antennae, and the legs, is pressed down on the surface of the leaf beneath the molted insect.

The molting period, as shown in tables 2 and 3, lasts from three to four days, being approximately of the same duration in both the red and yellow scales.

*Second Stage.*—After molting, the insect enters the second stage of its development.

That the first molt has been completed, is indicated externally by the extension of the wax rim, which is secreted around the margin of the former covering. On lifting this covering it is found that it may be removed readily from the insect, and remains more or less detached until the beginning of the second molt. It is during this stage that the scale coverings of the two sexes can be differentiated externally.

The female continues the rotating movement resulting in the addition of a circular rim to the covering of the first stage. This additional rim consists of a somewhat dull, compact, waxy secretion. Some interesting observations as to the secretion of this wax were made by placing a second-stage female, deprived of its scale covering, under a microscope and studying the flow of wax filaments from the abdominal pores. These filaments were at first excreted vertically, and upon attaining maximum length they fell over as a result of their own weight. They then became entangled in the pygidial fringe, because of the up and down movement of the pygidium. It appeared that the pygidial lobes and plates acted as the teeth of a comb, causing the tangled mass of wax filaments to be more closely united and formed into a ribbon which then passed out to form the waxy portion of the scale covering.

The early-stage male covering is found to be similar to that of the female except that the wax is lighter colored and does not seem to be of the same compactness and rigidity. Later, however, a decided elongation takes place primarily as the result of the cessation of the rotation of the body, and the continued secretion of the wax toward the posterior end of the body.

*Second Molt.*—The second molt is similar to the first. During this period, the female again becomes firmly attached to its dorsal covering, while the internal organs undergo transformation in preparation for the third or final stage. The coiled mouth parts are again evident, while the outer rim of the scale covering becomes clear-cut and well defined. Splitting or shedding of the old skin takes place along the sides of the body, the dorsal surface being incorporated in and firmly attached to the scale covering, while the ventral surface is pressed down on the leaf surface as in the case of the first molt. The duration of this molt was found to be about the same for both scales, the red showing an average of 3.63 days and the yellow 3.75 days.

During both the first and the second molt, there was a certain degree of mortality. This is evidently a result of the inability of the insect to break loose from its previous skin or the inability to uncoil its mouth parts so as to pierce the host tissue again.

*Adult Stage.*—Completion of the second molt brings the female to its final or adult stage. Before the production of young begins, two interesting subphases are to be noticed, which will be respectively called the prefertilization and the postfertilization stages.

Upon completion of the second molt, a further slow deposition of wax takes place until the external covering has attained its final dimensions. This stage can be easily recognized by the following characters:

1. Dorsal scale covering not firmly attached to the underlying female.
2. Slow rotating movement of body resumed in depositing wax rim.
3. New wax rim running around the margin of the older scale covering, soft and light gray in color.
4. On removing the dorsal scale covering, the exposed female is found to be pear-shaped, and in the case of *citrina* decidedly more yellowish in contrast to *aurantii,* which is more reddish.

It is during this stage that fertilization takes place, and the longevity of the insect depends upon how long after maturity fertilization occurs. Soon after fertilization has taken place the female enters its postfertilization stage.

During this stage the fertilized eggs develop rapidly. The outstanding changes that take place here can be briefly summarized as follows:

1. The rotation of the body ceases.
2. There is no further secretion of wax.
3. The body becomes firmly attached to the dorsal scale covering.
4. The body cuticula, especially on the dorsal side, becomes thickened, forming a fairly hard exoskeleton which is less elastic than in any of the previous stages. The cuticula is visible through the lighter wax area of the early third-stage wax rim. Together with the other previously incorporated molted skins, the dorsal scale covering takes on its characteristic color: brownish red in the case of *aurantii*, and yellowish green in the case of *citrina*.
5. When fully developed the thorax extends backward in a large rounded lobe on each side, projecting beyond the extremity of the abdomen, and giving the body a reniform shape.
6. The ventral portions of the molted skins of the previous stages are found closely pressed together on the surface of the leaf just below the adult female, and in comparison to those of the dorsal portions, are lighter, thinner, and more parchmentlike.

In many cases these ventral cast skins are found attached to the dorsal covering, with the result that it seems as if the female reposes within a baglike structure. This, however, is brought about after molting and is probably due to subsequent wax secretion, which glues the ventral skins to those of the dorsal covering. If this ventral scale is treated with KOH, the constituent skins fall apart, clearly indicating that the splitting of the skin during the previous molts had followed not only the general body margin, but often the margin of the lobes and plates as well.

The time that elapsed between the completion of the second molt and the appearance of the young was somewhat different for the red and yellow females; with the former this period averaged a total of 32.5 days and with the latter a total of 35.63 days, giving a difference of 3.13 days.

*Reproductive Period and Number of Young.*—Under a fairly high and constant temperature, 82° F, there was a continuous emergence of young over the whole reproductive period, which, under those conditions, lasted 60 days for both the red and the yellow species. The experiments were not extensive enough to furnish reliable data on the total possible number of young that might be produced, but they did show that both species produced young at about the same rate and in the same number.[6]

*Duration of Total Life Cycle.*—By comparing the respective life cycles, from free-moving larvae up to the time of reproduction, we find by referring to tables 2 and 3 that the cycle of the yellow runs from 61 to 68 days, and that of the red from 60 to 63 days, giving an average of about 65 and 61 days respectively, or a difference of about 4 days.

*Sex Ratios.*—Of the 521 red-scale adults procured during the second half of the year there were 319 females and 202 males. Of the 249 yellow adults procured during the same period there were 108 females and 141 males. One hundred red larvae and 70 yellow larvae were transferred during the early spring. Of the 65 red scales which reached maturity there were 19 females and 46 males. Of the 48 yellow scales which reached maturity, the females were even more decidedly in the minority, there being 2 females and 46 males. The preponderance of males in the last case is unaccounted for.

[6] The number of young produced by a single female varies greatly according to the food and temperature conditions, and to the technique employed by those engaged in conducting the experiments. One hundred and fifty young were produced by one female on a lemon fruit. There seem to be more young produced on the fruit than on the leaves.

## BREEDING EXPERIMENTS

Since parthenogenesis has been reported for various members of the Coccidae, a series of experiments was conducted to determine whether or not this might be the case with either *citrina* or *aurantii*.

Two lots of 50 red and 50 yellow free-moving larvae were placed upon previously banded leaves of two young potted orange trees. Each of these trees was then isolated in a sealed, insect-proof cage, so as to prevent any outside males from reaching the females. As soon as sex differentiation was evident all the males were removed, leaving a total of 30 red females and 35 yellow females in each cage. These females were kept thus isolated until most of them were dead, and in no case were any progeny produced, showing that parthenogenesis did not take place.

The active part which the male plays in fertilization was further brought out in a number of instances where the act of copulation was observed. The following notes were made in one case: Just after emerging from beneath its scale covering, the male moved actively around for some time until it eventually stopped in front of an early third-stage female, at which time copulation took place. The male withdrew a short distance, and began to wipe off its antennae with the aid of its fore legs. This process was kept up for about ten minutes while the movements became gradually slower and slower until all indications of life ceased. This observation, that is, from the time the male emerged until its death, lasted from 9:45 A.M. to 10:35 A.M., indicating the short life of the adult stage of the male.

In a number of cases the males took to flight immediately upon emergence, evidently seeking to fertilize females on adjacent leaves. Flight of the males was sluggish, taking place in gradually increasing circles. In no instance did a male fertilize more than one female, although the same female was fertilized by two or more males. The larger proportion of males appeared during the night, a condition to be expected as the male is of an extremely soft and frail nature, and could not survive exposure to direct sunlight and high temperatures.

A number of experiments were conducted with the object of determining whether or not interbreeding takes place between *aurantii* and *citrina*. As time would not permit more extensive investigations, the results presented here cover only one or two life cycles.

Because of their delicate nature and extremely short life, the males proved very difficult to handle and control in large numbers. Other methods eliminating direct handling had to be resorted to, and of these,

three types will be described. Insect-proof cages were used throughout so as to prevent any outside males from coming in contact with the females used in the experiments. A quarantine isolation room was used when it was necessary to remove any of the males or females.

*Experiment A.*—Six leaves of a young potted orange tree were cleaned and their petioles banded with paraffin wax. Leaves 1, 3, and 5 were each colonized with 50 active red-scale larvae, and leaves 2, 4, and 6 with yellow-scale larvae. As soon as the sexes could be distinguished, the red males and the yellow females and the paraffin bands on the petioles were removed. In other experiments of the same type the red females and the yellow males were removed. To serve as checks, only the males of one type of scale were removed, leaving both the males and females of the other type. The results of these experiments are briefly indicated in table 4.

TABLE 4

RESULTS OF INTERBREEDING EXPERIMENTS

| Experiment | Matings | Results |
|---|---|---|
| A 1 | R♀ × Y♂ | No progeny produced |
| A 2 | Y♀ × R♂ | No progeny produced |
| A 3 (Control) | R♀ × Y♂<br>Y♀ × Y♂ | Progeny from Y × Y only, none from R × Y, indicating crossing only between yellow females and yellow males |
| A 4 (Control) | R♀ × R♂<br>Y♀ × R♂ | Progeny from R × R only, none from Y × R, indicating crossing only between red females and red males |

*Experiment B.*—Four small trees were alternately colonized as follows: trees 1 and 3 with red larvae, and trees 2 and 4 with yellow larvae. The males in all four cases were removed as soon as this was possible. Oranges infested with large colonies of healthy yellow scales were placed in the cages containing trees 1 and 3, in such a manner that while the males could escape and reach the red females, the young active yellow larvae from the fruit were unable to reach the trees. With trees 2 and 4 the same was repeated, using fruit infested with red scale. In no case were young produced, indicating that no interbreeding had taken place.

*Experiment C.*—Colonization of four small trees with red and yellow larvae was duplicated as in Experiment B. Later when sex differentiation was possible, the following removals were made:

(1) All red females from tree 1, leaving red males.
(2) All yellow males from tree 2, leaving yellow females.
(3) All red males from tree 3, leaving red females.
(4) All yellow females from tree 4, leaving yellow males.

Trees 1 and 2 were then caged together, and similarly trees 3 and 4, thus allowing ample opportunity for any interbreeding to take place if such were possible. However, as in the two former experiments, no interbreeding took place.

## METAMORPHOSIS OF THE MALE

*First Stage.*—The metamorphosis of the Diaspine scales is more anomalous than that of any other insect. During the first nymphal stage the two sexes are indistinguishable. Both possess legs, antennae, and eyes, all of which are lost during the first molt. The female then continues with its gradual metamorphosis while the male follows an altogether different development, very similar to a complete metamorphosis. After its second molt, the male loses its functional mouth parts and gradually acquires a new set of legs, antennae, and wings. The legs and antennae are derived from histoblasts, or imaginal disks, which are similar to those found in insects with a complete metamorphosis, while the wings are developed externally, indicating a gradual development. The type of metamorphosis which the male undergoes is, therefore, neither strictly complete nor gradual.

The external characters of the four different instars of the male of *Aonidiella aurantii* have been fully described by Quayle[28] and Berlese and Leonardi,[1] and as the descriptions of these authors are identical to those of the writer for *citrina*, the following discussion will be limited to the development of the male appendages. In no instance was the presence of histoblasts noticed in the first-stage larvae, probably as a result of the expected minuteness of their formative cells during this stage. Matters were further complicated as there was no way of determining whether a male or female larva was under examination. The second stage of the male will, therefore, be taken as the starting point of the internal development of the appendages.

*Second Stage.*—About two days after the first molt the histoblasts of the antennae and the legs are noticeable (fig. 1*A*). The antennal histoblasts are situated at the extreme anterior portion of the cephalic end of the body, while those of the legs are found in the ventral thoracic region. These histoblasts first appear as thickenings of the hypodermis and later take on the form of round pockets or buds. Each bud forms the

center of a so-called peripodal cavity, which, as development proceeds, eventually opens, while the bud itself evaginates, sending out a finger-like process. These processes which represent the rudimentary appendages, point anteriorly in the case of the antennal and fore-leg histoblasts, and posteriorly in the case of the middle and hind-leg histoblasts. The wings gradually arise from buds on the lateral margins between the dorsal and ventral surfaces of the body.

The first pair of accessory eyes is situated posterolateral of the antennal buds and the second pair is found on the ventral cephalic surface, opposite each end of the brain. During this stage the eyes are present as dark-violet diffused areas. Just before molting, the appendages have attained approximately one-third of their final length. At about the same time the thoracic muscles begin to show plainly on the dorsal surface.

Transformation into the following or prepupal stage takes place rapidly so that just prior to molting it is found lying within the body wall of the second stage and separated from it by a margin filled with liquid. Actual molting takes place by a contraction, followed by a sudden expansion of the new internal prepupal body, causing the cuticula of the second stage to split at the cephalic end and to be pushed backward toward the abdomen where it can often be seen projecting from underneath the scale covering. During this molt the mouth parts are lost and not replaced during the subsequent stages.

*Prepupal Stage.*—The prepupal stage (fig. 1*B*) is marked by the further development of all the appendages. The latter are clearly seen enclosed within a thin sheath, with the joints, indicated by cross divisions, becoming more and more apparent. Schmidt,[30] working on the male of *Aspidiotus nerii,* was of the opinion that the primary object of this stage is to supply the necessary material at the right place, and to make, through molting, sufficient room for the subsequent stages. The body gradually takes on the shape of the final stage, and in addition, the histoblast which, upon evagination, will give rise to the genital organ or style, is clearly seen toward the tip of the last abdominal segment. At the end of this stage, the appendages attain approximately two-thirds of their final dimensions. The molting process is similar to that of the second stage.

*Pupal Stage.*—During the earlier portion of the pupal stage (fig. 1*C*), the joints of the different appendages become clear-cut; the lighter divisions marking the segments is not clearly seen until shortly before the final molt. The bristles on the legs and antennae are difficult to detect at first, but become clearer and better defined with the formation of the final cuticula. The evaginated style and all the other appendages are

clearly seen enclosed within their thin, chitinous sheaths; the wings are collapsed and folded within their sacs. The eyes have changed from diffused areas to definite areas which they occupy in the adult male. The last or final molt produces the fully developed male, which remains under the shelter of its scale covering for a day or so longer before emerging.

## EXTERNAL MORPHOLOGY

Because of its economic importance and wide distribution throughout the citrus-growing sections of the world, *Aonidiella aurantii* has attracted the attention of many workers, and the external characters of the female have been described in detail more than once. With reference to *citrina,* no separate description has been made, as workers generally have taken the latter to be structurally identical to *aurantii.*

However, to acquaint the reader with the female structures upon which the identification of this species is based, and in order to facilitate a better understanding of those characters which, in the course of this study, were found to be specific and constant for *citrina* but not for *aurantii,* a résumé will be given, embodying the original description of Maskell[22] and also the subsequent ones of Comstock[5] and Brain.[2] No constant morphological differences could be detected in the immature stages of either of the two scales, and as these were fully described by Quayle,[28] further descriptions will be omitted in this discussion. Additional notes on the adult male of *citrina,* and its development, will be given under a separate heading.

For the study of the external chitinous structures, the standard methods described by Gage,[10] Ferris,[9] and Brain[2] were employed; of these the 10 per cent KOH treatment and subsequent staining with either acid or basic fuchsin proved the most satisfactory. Rapid examinations of unstained and live specimens were made by mounting in glycerin. All measurements were made with the aid of a standard eyepiece micrometer.

*Adult Female of Aonidiella Aurantii.*—The scale covering is nearly circular, slightly broader (1.9 mm) than long (1.77 mm), with thin, flat margins and the central area flatly convex, and generally appears shiny or polished. The reddish color is due to that of the female insect beneath the scale. The exuviae are regularly central, and are covered by a thin layer of secretion. A small prominent spot with a concentric ring of whitish waxlike substance is found in the center of the larval exuvia. The ventral exuvia consists of the lower half of the molted skins of the previous stages; however, it varies greatly in texture, being of a grayish

white, parchmentlike character, often fused with the inner surface of the dorsal scale covering.

The body is orange red. It is reniform in shape as a result of the lateral margins of the body, which extend backward as two large rounded lobes, one on each side of the retracted abdomen. The body is flat beneath and slightly convex above. The abdomen is terminated by a flattened chitinous region or pygidium, composed of several fused segments. Antennae are absent, except for two minute papillae or antennal tubercles, set well back from the mouth parts and each carrying a fine, slightly curved spine. The derm is membranous throughout, except for the pygidium.

The pygidium (figs. 2*C* and 2*D*) is broadly rounded. The anus is on the dorsal side of the pygidium, and the genital aperture on the ventral side. On each side of the dorsal median line, about 17 tubular ducts are found; their openings are arranged more or less in three lines. Circumgenital glands are absent. Green [11, 12] has directed attention to the possible connection between the circumgenital glands and oviposition. Thus in those species which are viviparous, the glands as a rule are wanting, while they are present in all oviparous species, as stated by Imms.[14]

On the pygidial fringe there are three well developed lobes: L1, L2, and L3. L1 and L2 usually are distinctly notched on both margins, the notch on the outer margin of L2 being more pronounced. The notch on the mesal margin of L1 is often nearer the distal end of the lobe than that of the outer margin.

The plates are found between the lobes, which they exceed in length. They are arranged as follows:

There are two plates between the median pair of lobes (L1–L2). These are deeply fringed on their distal margins only.

There are two plates between lobes L1 and L2, two between L2 and L3, and three between L3 and the margin of the body. These are all fringed on their outer margins.

The first plate laterad of the second lobe, and the three plates laterad of the third lobe, are each deeply bifurcated, each bifurcation being fringed on the outer margin.

*Adult Female of Aonidiella Citrina.*—The scale covering is similar in structure and outline to that of the red-scale female, except that it is slightly flatter and smaller, having an approximate width and length of 1.725 mm and 1.58 mm respectively. (For further comparative dimensions, see table 5.) The most outstanding difference, however, is the yel-

## TABLE 5

COMPARATIVE MEASUREMENTS OF ADULT FEMALE SCALE COVERINGS; MILLIMETERS

| *Aonidiella aurantii* | | | | *Aonidiella citrina* | | | |
|---|---|---|---|---|---|---|---|
| Entire scale covering | | Longest diameter of second-stage incorporated skin | Longest diameter of first-stage incorporated skin | Entire scale covering | | Longest diameter of second-stage incorporated skin | Longest diameter of first-stage incorporated skin |
| Width | Length | | | Width | Length | | |
| 2.025 | 1.800 | 0.810 | 0.405 | 1.800 | 1.575 | 0.720 | 0.315 |
| 1.800 | 1.710 | .720 | 405 | 1.575 | 1.485 | .675 | .315 |
| 1.935 | 1.820 | .810 | .360 | 1.710 | 1.575 | .675 | 315 |
| 1.890 | 1.820 | .810 | .360 | 1.800 | 1.575 | .675 | .360 |
| 1.800 | 1.800 | .810 | .360 | 1.575 | 1.575 | .675 | .360 |
| 1.935 | 1.800 | .810 | .405 | 1.820 | 1.530 | .720 | .360 |
| 1.935 | 1.890 | .855 | .360 | 1.935 | 1.710 | .765 | .360 |
| 2.025 | 1.710 | .810 | .405 | 1.665 | 1.575 | .720 | .315 |
| 1.890 | 1.890 | .765 | .405 | 1.800 | 1.710 | .720 | .360 |
| 2.025 | 2.025 | .810 | .405 | 1.800 | 1.800 | .765 | .405 |
| 1.890 | 1.800 | .810 | .360 | 1.710 | 1.350 | .630 | .405 |
| 1.710 | 1.485 | .810 | .405 | 1.820 | 1.665 | .810 | .315 |
| 1.890 | 1.665 | .810 | .405 | 1.575 | 1.350 | .630 | .360 |
| 2.025 | 1.890 | .810 | .405 | 1.665 | 1.575 | .765 | .405 |
| 1.890 | 1.575 | .810 | .315 | 1.710 | 1.575 | .765 | .360 |
| Av. 1.900 | 1.777 | 0.800 | 0.384 | Av. 1.725 | 1.581 | 0.714 | 0.354 |

## TABLE 6

COMPARATIVE MEASUREMENTS OF PYGIDIAL LOBES; MILLIMETERS

| *Aonidiella aurantii* | | | *Aonidiella citrina* | | |
|---|---|---|---|---|---|
| L1 | L2 | L3 | L1 | L2 | L3 |
| 0 011 | 0 011 | 0.015 | 0.015 | 0.015 | 0 015 |
| .015 | .015 | .015 | .019 | .015 | .015 |
| .015 | .015 | .011 | .023 | .019 | .019 |
| .011 | .015 | .015 | .023 | .019 | .019 |
| .015 | .011 | .015 | .019 | .019 | .019 |
| .015 | .015 | .015 | .019 | .019 | .019 |
| .015 | .015 | 011 | .023 | .023 | .023 |
| .015 | .011 | .015 | .019 | 015 | .015 |
| .015 | .015 | .015 | .023 | .019 | .019 |
| .015 | .015 | .015 | .019 | .019 | .019 |
| .015 | .015 | .011 | .021 | .019 | .019 |
| .015 | .011 | .015 | .019 | .019 | .019 |
| .015 | .015 | .015 | .019 | .019 | .019 |
| .015 | .015 | .011 | .019 | .019 | .019 |
| Av. 0.014 | 0 014 | 0.014 | 0.020 | 0.018 | 0.018 |

low color of the covering, which differentiates it easily from that of the red scale.

The body is identical in shape to that of the red female, but different in its clear, yellowish-green color.

On the pygidial fringe (fig. 2*A*), lobes L1, L2, and L3 are well developed. Laterad of the last three plates, the margin of the fourth abdominal segment forms a well-defined triangular process or lobe. The surface of the latter varies from serrate to smooth, as does that of the other lobes. This lobe was found to be constant for a large number of individuals examined, thus permitting mounted specimens to be differentiated.

Considering the three main lobes, the writer's attention was drawn to their evident slender appearance. Measurements were taken which showed them to be appreciably longer than the corresponding ones of the red females. Table 6 presents measurements taken at random of 14 red and 14 yellow females. Furthermore, as shown in figure 2, lobes L2 and L3 of the yellow females are not so deeply notched as the corresponding ones of the red females.

The structure, location, and bifurcation of the plates are similar to those of the red scale. The pair of plates situated between the median pair of lobes (fig. 2*B*) appear more fringed than those of the red scale. However, as a result of a great variation within the individuals studied, no great importance was attached to this difference.

*Adult Male of Aonidiella Aurantii and Aonidiella Citrina.*—In addition to the studies conducted on the females of *Aonidiella aurantii* and *A. citrina,* special attention was also given to the external morphology of the males of both of these species. The object was to determine whether or not there were sufficient constant differences by which the two species could be segregated. A careful examination failed to show any differences upon which a satisfactory and absolute differentiation could be based. However, as a result of these studies, additional data on the external morphology of the male of *Aonidiella citrina* (fig. 3) were obtained which will be briefly presented in the following pages.

The general description given by Quayle[28] for the male of *Aonidiella aurantii* will cover equally well that of *A. citrina.* Quayle states:

> The adult male has a wing expanse of 1.5 mm.; length exclusive of style .6 mm.; style .22 mm.; color orange yellow; antennae ten-jointed, the first two segments being much shorter and thicker than the others. The comparative lengths beginning with the proximal one, are as follows: 5–4–17–20–20–20–18–15–13–17. Total length 0.5 mm. The antennae are light colored with some yellow pigment. On all the joints excepting the first two are rather long hairs. The lateral pair of eyes are dark brown

and situated just laterad of the antennae. The ventral pair of eyes are much larger and closer together and situated more posteriorly. The legs, excepting coxae which are yellow, are glassy white, and the tarsi light brown. The thoracic band is of a light brown color. The halteres are club shaped, with the slender hook arising from the tip of the club.

As the above description is rather brief, the following additional descriptions of the cephalic, thoracic, and abdominal regions are given. By reference to figures 3, 4, 5, and 6, the more important structural characters of these can be easily followed. Some of these structures are named according to homologous ones worked out by Berlese and Leonardi[1] for *Aspidiotus hederae*.

Figure 4 represents a semidiagrammatic aspect of the ventral side of the *head;* the dorsal structures are indicated by broken lines. The head is more or less triangular in outline with the anterior end somewhat compressed dorso-ventrally. It is broadest across the posterior or basal end, which is marked by a fairly well defined suture.

On each side of the head and well within its margin, the malar ridges start at a point intermediate between the dorsal accessory and the ventral accessory eyes and the primary eye, and extend in a posterior direction. These malar ridges are strongly chitinized, slightly curved, broadest through middle, and tapering toward each end. In the immediate vicinity of the basal suture, each turns directly inward and upward and fuses with its corresponding mate from the opposite side on the median line of the head, thus giving rise to a ridge which is known as the ocular apophysis. The latter has a slightly broadened apex and terminates in the region between the two ventral accessory eyes.

On the dorsal surface of the head, situated between the dorsal accessory eyes, the peculiarly shaped cephalic ridge is found. Situated at the base of the ocular apophysis, a fleshy tubercle, representing the rudimentary mouth parts, is found. In cross sections the esophagus terminates in this tubercle, showing no actual opening to the outside. Viewed ventrally, each of the dorsal accessory eyes is concealed by a ridge or scape, which extends posteriorly from the base of the first antennal segment. The bases of the antennae are separated by a row of well defined and unpaired spines which are situated in a shallow furrow.

As pointed out before, there are two pairs of accessory *eyes* and one pair of primary eyes. The ventral accessory eyes appear as two large, ovoid, closely approximated areas situated on each side of the median line in the caudal half of the ventral aspect of the head. The circular and dark-brown dorsal accessory eyes lie just caudad of the base of the antennae on the dorso-lateral line of the head. The primary eyes can be

seen best from the ventral side. They are two colorless, beadlike projections with translucent tips, situated on the lateral surface of the head, opposite the cephalic half of the ventral eyes, and immediately posterior to the dorsal accessory eyes. It is remarkable that the primary eyes can best be seen from the ventral side, even though they lie just behind the dorsal eyes.

The dorsal and ventral accessory eyes are similar in structure (fig. 1*D*). Each eye is surrounded by a groove which is formed as the result of the pushing upward of the body wall into a ridge. The lens of the eye is a large, spherical, globular, transparent body, consisting of a homogeneous substance covered by a thin layer of chitin. Between the lens and the visual rods is the corneal hypodermis, which occurs as a thin flattened layer. The iris surrounds the corneal hypodermis and consists of a layer of highly pigmented cells. Next to the corneal hypodermis is a crescent-shaped area filled with visual rods. The latter are translucent and slender, tapering slightly toward their distal ends, and separated from one another by a dark seam. The retina which follows is made up of retinal cells regularly arranged opposite the bases of the visual rods. The distal ends of the retinal cells are contracted and prolonged into nerve fibers which unite to form the optic nerve.

The primary eyes are very simple in structure and differ from the accessory eyes in that they do not possess the corneal hypodermis, iris cells, and visual rods. The lens consists of a slight thickening of the cuticula with an outer convex surface.

The *prothorax* (fig. 4) is mostly soft and fleshy, as no sclerites are developed. In each of its lateral ventral halves there is a large and well developed trochanter which articulates posteriorly with the first pair of coxae.

Of the three thoracic regions, the *mesothorax* (figs. 5 and 6) is the largest, showing a well developed tergum, pleuron, and sternum. Figure 5 represents a dorsal aspect of the mesothorax.

The scutum occupies practically half of the entire mesothoracic area. Anteriorly it is bounded by a chitinous ridge which on its inner side, forms a loop enclosing a humplike area, called by Berlese and Leonardi[1] "gobba del mesotorace"—the prominence of the mesothorax. Posteriorly the scutum is separated from the scutellum by the broad, dark-brown thoracic or interscutellar band.

The posterior half of the mesothorax is occupied by the heart-shaped scutellum. The pleuron consists of circular sclerites with the wings and halteres arising between the tergo-pleural sutures.

Figure 6 represents a ventral aspect of the mesothorax. The mesosternum appears like a chitinous rectangular frame with an internal fork-like structure, the function of which is evidently to serve as a means of attachment for the thoracic muscles. A chitinous ridge or linear sternite is found on the median line of the prosternum.

The *metathorax* (fig. 6), like the prothorax, is fleshy and poorly defined, especially in specimens treated with KOH. On the ventral side of the metathorax two long chitinous processes, called the epimera by Berlese and Leonardi,[1] run posteriorly between the bases of the halteres and the third pair of coxae. The second pair of coxae are attached to similar but shorter structures from the pleural regions. The position of the two pairs of spiracles in the mesothorax and the metathorax is also indicated in figure 5.

The *abdomen* (fig. 3) consists of eight segments and is terminated by the prominent style or genital sheath, which encloses the aedeagus.

*Mouth Parts of Female.*—The gross arrangement of the mouth parts (figs. 7*B* and 7*C*) of *Aonidiella aurantii* and *A. citrina* is homologous to the corresponding arrangement of the generalized homopteran, and does not show any marked differences from the Coccidae worked out by Mark,[21] Putnam,[27] Moulton,[23] Berlese and Leonardi,[1] and Childs.[3] The mouth parts are essentially adapted for piercing and sucking, the food being entirely liquid.

In this discussion, the descriptions will be limited almost entirely to the more important parts which have a direct bearing upon the structure and function of the mouth parts.

The chitinous, boxlike framework (fig. 7*B*) is clearly visible on the ventral median surface of the female, especially in those individuals which have been treated with KOH and subsequently stained. Its function in the main is twofold: First, it is very important in serving as a means of attachment for the powerful muscles which govern the pharyngeal sucking and salivary apparatus. Second, it protects and supports the underlying organs.

The framework is broadly triangular in shape, as a result of four pairs of chitinous arches which enter into its construction. Its base is formed by a smaller dorsal and larger ventral portion, called respectively by Mark,[21] the "arcus-superior" and the "arcus-inferior." The portions which form the laterals of the triangle have been called by the same author, the "costae"; the dorsal ones, costae superiores, and the ventral ones, costae inferiores. These structures are fused together at their extremities, forming the rigid boxlike structure. At the apex which points

posteriorly, sufficient space is left for the passage of the buccal setae, pharynx, etc.

There are four extremely long, slender, bristlelike setae (fig. 7*B*), representing the two pairs of modified *mandibles* and *maxillae*. Together they form the rostralis, in the formation of which the maxillae play the more important role. The two maxillae are closely united, leaving a small lumen which serves as a passage through which the liquid food from the host to the pharynx may pass; on their exterior they are protected by the mandibles, situated on each side. At its proximal part, each seta gives rise to a conical body, called the "conus" by Mark.[21] Distally each pair passes through the anterior opening of a curious, highly chitinized, baglike apparatus, called the clavus. The clavus gradually tapers, forming, with its corresponding arm from the opposite side, a common apex. Within this apical portion, the two pairs of buccal setae come in close contact with one another, giving rise to the rostralis. The rostralis runs posteriorly, making a loop within the crumena. During the larval stage, the crumena is extremely well developed, extending as far back as the second abdominal segment. In the case of *Warajicoccus* Kitao[15] describes the crumena as follows:

> A tubular passage, which extends back just below the thoracic ganglion and terminates in a blind sac, embedded in the connective tissue without lying free in the body cavity, as stated by some writers. In cross section it is dumb-bell shaped, its wall consisting of three layers, much as in the integument. The inner layer, exclusive of its innermost part, is fairly thick and weakly chitinized. Next comes the middle hypodermal layer which is very thin. The outer layer is represented by an inconspicuous basement membrane.

From the crumena, the rostralis passes upward and so through the rostrum. Just before birth, and during the subsequent molts, the mandibles and maxillae are found to be coiled up, a pair on each side of the brain. The mandibles and maxillae evidently arise from their respective imaginal disks; the latter lying beneath that of the former. These disks are of hypodermal origin, forming, during each molt, a new set of mouth parts.

The *rostrum* (fig. 7*C*) is but a modified, one-segmented labium, conical in shape and attached at its base to the ventral side of the thorax. The lower side of the rostrum forms, as a result of folding, a closed canal in which the setae lie. Within the rostrum and attached to chitinous plates are a few muscles, undoubtedly used when the setae are forced into the host tissue.

The *salivary pump* (fig. 7*C*) lies beneath the anterior part of the pharynx and close to the point of junction of the two arms of the clavus. It

consists of a highly chitinized, cuplike structure or cylinder, carrying an inverted top in the form of a "plunger." This plunger is flexible, with a heavy, chitinized, Y-shaped tendon, to the base of which are attached some powerful retractor muscles. There is no doubt that these muscles cause the rapid up and down movement of the plunger. A small flaplike membranous ridge runs around the middle of the plunger. The function of this flap or ridge is valvular, cutting off, on the down stroke of the plunger, the flow of saliva from the glandular duct.

The paired salivary branches unite to form a common duct, which enters the cylinder through the anterior part of the dorsal surface. Posteriorly the lateral sides of the cylinder curve inward, leaving a small lumen, which communicates with the general mouth cavity. The latter is bounded by the lingua (arcus superior) and the labrum (arcus inferior), close to the extremities of which the bases of the mandibles and maxillae are found.

There seems to be some doubt as to the actual function of the salivary pump. In the case of certain heteropterous forms, studied by Muir and Kershaw,[24] its mode of operation is described as follows:

"The plunger being retracted by the muscles, draws the saliva from the ducts into the syringe-barrel. On relaxation of the muscles, the natural elasticity of the plunger performs the return stroke, closing the valve and forcing the saliva past the tongue on the base of the labium."

Berlese and Leonardi,[1] who studied several species of coccids, is of the opinion that the pump draws the saliva from the large paired glands, instead of only from their common duct. Childs,[3] on the other hand, states that:

> The rule for most insects with comparable organs (large paired glands, ducts, and openings into the cylinder) is that these glands, as a result of an internal pressure caused by a continual secretion of the cells from within, or from muscular action, force the juices out. In the case of *Epidiaspis piricola*, no muscles are to be found that could perform this function, and, from the make-up of the glands themselves, they seem to the writer to be admirably adapted to operate through a pressure formed from within. Again, from the make-up of the long, slender, four-pieced proboscis, it would seem to be impossible to pump saliva into the plant tissues, for pressure from the inside would disrupt the tube. Necessarily, therefore, if there is a passage of fluid down this setal arrangement it would have to be done with little or no pressure.

As a result of a number of experiments conducted upon live specimens, the observations of the writer seem to agree more with those of Muir and Kershaw than with those of Childs. By careful manipulation, so as not to damage or break off their rostrali, early third-stage females were removed from the host tissue and placed upon slides directly under

the microscope. Immediately, a clear, colorless drop of saliva was formed at the distal end of the rostralis, indicating that saliva does pass down the latter organ and so into the tissues of the host. That the food juices pass up and down separate canals within the rostralis was further demonstrated by placing the tip of the latter in a drop of water-soluble stain. The stain was found to pass up the rostralis, while the continued flow of saliva was indicated by a clear zone within the drop of stain.

By taking into consideration the length and chitinous nature of the rostralis, its position within the host tissues, and the rapidity with which the saliva passes down its whole length, one must come to the conclusion that a certain amount of pressure is derived from the highly developed salivary pump. Passage of the saliva from the glandular cells into the ducts is probably caused, as has been suggested, by an internal pressure, the saliva being drawn further into the cylinder by the upward stroke of the plunger. Disruption of the setal tube within the plant would hardly result from internal pressure, as any such tendencies would be sufficiently equalized by the surrounding tissues. The toxic action of the saliva of this species upon the chlorophyl of the host leaf is clearly indicated by the appearance of a yellow streak, running from underneath the side of the settled insect.

The *pharynx* (fig. 7*C*), which opens into the mouth cavity, is somewhat swollen and provided with thick, irregular, chitinous walls, which abruptly thin down as they come to form the walls of the true esophagus. To these thick walls, called "uva" by Mark,[21] are attached a series of powerful divaricator muscles passing through the labrum, with their other extremities attached to the arcus inferior. The function of these muscles is to contract and expand the pharynx, thereby causing the liquid food to be sucked up through the rostralis and mouth cavity and so into the esophagus.

## INTERNAL ANATOMY OF AONIDIELLA CITRINA

Morphologists have neglected the Diaspine group in the past, directing their studies more particularly to the generalized or unarmored coccids. Berlese and Leonardi[1] and Childs[3] are the only workers who have contributed outstanding papers on the internal anatomy of the Diaspidinae. The former worked on *Lepidosaphes vulva*, and the latter on *Epidiaspis piricola*. It is hoped, therefore, that the following observations, although not agreeing in all respects with those of the above-mentioned authors, will contribute something to a better understanding of the internal structures of this very interesting group.

For the investigation of the internal anatomy the usual methods of serial sections were employed. Sections varying from 3$\mu$ to 10$\mu$ were cut and subsequently stained on the slide. For staining, both Delafield's haematoxylin and Mayer's haemalum were used, with eosin as a counterstain. For sectioning material, early third-stage females were preferred to the adult or full-grown females, because the latter were found to tear considerably as a result of their hard, inseparable scales, chitinous cuticula, and the resistance offered by the fully developed ovaries. The large ovaries with their developing embryos also tended to obscure the other finer anatomical structures. The majority of these difficulties became negligible with early third-stage females, as their scale coverings could be removed with ease, thus leaving only a soft cuticula with which to contend. The ovaries were still in a developmental stage, and were thus not sufficiently tough to offer any difficulties in cutting. Direct dissections were also attempted, but again the fan-shaped reproductive organs occupying the greater part of the body cavity proved the greatest hindrance in making such dissections a success. The majority of the drawings were made with the aid of a camera lucida.

*Integument.*—The structure of the integument is similar to that of most insects. The whole body is covered by a cuticula which, in well stained sections, shows two layers, namely, the epidermis and the dermis, the former being much thinner than the latter. Next beneath the cuticula is the hypodermis which rests upon an extremely thin and hardly noticeable basement membrane. The hypodermis forms a continuous layer of cells which change from a columnar type in the median part of the body, to a fusiform type at both the anterior and posterior extremities.

*Digestive System.*—Of all sucking insects, perhaps the Homoptera offer the greatest variation in the form of their digestive systems, most of them showing an organization which greatly attracts the interest of the research worker. Even within the Coccidae we find the digestive system diverging from a more or less straight duct, as in the Disapidinae, to those which assume a convoluted or recurrent course as has been demonstrated for members in the other subfamilies such as the Lecaniinae, Xylococcinae, Coccinae, etc.

Passing anteriorly from the mouth cavity, the *pharynx* runs into the thinner walled esophagus (figs. 7*C* and 8*B*), which extends further forward until it reaches the base of the cephalic ganglion. It then curves sharply backward, running between the circumesophageal commissures into the thorax where it joins the ventriculus. At the point of junction, the cardiac valve is found. The esophagus is narrow and of more or less the same width throughout. Its wall consists of a fairly thick, chitinous

intima and an epithelium carrying the esophageal glands in the region where the esophagus curves toward the ventriculus.

Berlese and Leonardi[1] state that the ventriculus or *stomach* is a closed sac in *Lepidosaphes vulva,* entirely disconnected from the hind intestine except for two extremely thin and ductless ligaments which stretch from the posterior end of the stomach to the anterior end of the rectum. Digestion, according to them, takes place as a result of the action of the digestive juices rendering the food contents capable of passing by osmosis into the main haemocoelic cavity; the waste substances are removed from the latter by means of the greatly enlarged Malpighian tubes.

Childs,[3] on the other hand, in discussing the relation of the stomach to the hind intestine in *Epidiaspis piricola,* gives the following inadequate description:

"However (in referring to the findings of Berlese), the writer finds some sections in his series that show what can hardly be denied to be direct connections."

As no further information was available from the rather indistinct illustrations, the writer is still at a loss to explain what Childs referred to as "direct connections"; that is, whether they occur between the hind intestine proper or the Malpighian bulb, a structure which will be described later.

From the respective findings of these two authors, it is evident, therefore, that they are not in agreement on a morphological point, which is of sufficient importance to divide the Diaspine digestive system into two separate types, depending on whether or not a connection exists between stomach and hind intestine. It is possible that certain minor variations do occur in the different species studied by these authors, but considering the digestive system as a whole, one is inclined to believe that the Diaspidinae possess a constant type peculiar to this group. In the discussion which follows it will be shown that a direct connection does exist between the fore and hind intestine.

In *citrina,* as a result of a constriction in the wall of the anterior part of the ventriculus (fig. 8*B*), there is found around the cardial valve, a small antechamber, the function of which is probably comparable either to that of a sucking stomach or a filter chamber. Posteriorly the stomach widens, taking on the characteristic baglike appearance. The digestive epithelium of the stomach consists of a row of irregular cells, with an occasional one greatly enlarged. These large cells, carrying correspondingly large nuclei, are probably the active, secreting ones, while the smaller ones are still in a developmental stage. These cells are all poorly

demarcated and void of any protrusions into the general lumen of the stomach, giving the surface of the enteric epithelium a smooth and wavy appearance. This type of structure immediately suggests, in part at least, Berlese's osmotic theory of digestion and assimilation. The outer ends of the epithelial cells rest upon an extremely thin basement membrane followed by the muscular layers.

At its ventral posterior end (fig. 8*C*), the ventriculus is found to be definitely connected with a highly nucleated, ovoid body called the Malpighian bulb (bulbo del peduncolo dei Malpighiana) by Berlese and Leonardi.[1] Viewed from a basal aspect this bulblike body, or swelling of the small intestine, is easily recognized not only by its nucleated appearance, but also by its median position above the junction of the two Malpighian tubes. From this aspect the body also seems to be actually separated from the stomach, a condition which probably led Berlese to believe that it had no connection with the stomach. The actual connection, however, is not readily detected because it occupies only a small area between the anterior-ventral surface of the Malpighian body and the stomach. The junction of the two well developed Malpighian tubes at this point also tends to obscure a clear view of the connection proper. In sections the lumen of the connection appears to be filled with a porous protoplasmic tissue, as a result of the proximity of the epithelium of the stomach and the Malpighian bulb.

That digestive activity does take place within the Malpighian bulb, is indicated by its well developed epithelial layer which consists, as in the stomach, of irregular, nucleated cells, the outlines of which are not clearly defined. A short, narrow and slightly curved duct leads from the posterior end of the Malpighian body, connecting the latter with the anterior extremity of the rectum. This duct is readily recognized by its profusely nucleated cells which are closely united around a well marked lumen.

The *rectum* (fig. 8*A*), projecting posteriorly, is a comparatively wide and straight passage lying in the median portion of the body. It gradually tapers down, assuming the character of a narrow canal which shortly opens to the exterior through the anus situated on the dorsal side of the pygidium. On one side there is a caecum which was at first mistaken for a rectal fold. Kitao[15] mentions a similar structure in the case of *Warajicoccus*. Structurally the rectum is very simple, presenting only a thin epithelium, the cellular structure of which is extremely hard to differentiate.

There is only a single pair of large, cylindrical *Malpighian tubes* (figs. 7*A* and 8*C*) lying in the main body cavity. From their point of fusion

between the stomach and the hind intestine, the tubes run laterally for a short distance, turn abruptly upwards and then run posteriorly, almost to the caudal end of the body. Their posterior ends are attached to the sides of the rectum, a short distance forward of the anal aperture, by thin filaments. Structurally the tubes consist of large, polygonal cells, and have reticulated cytoplasm and distinct nuclei. These cells stand in conjunction with a central threadlike lumen which runs anteriorly, ultimately to open into the small intestine between the stomach and the Malpighian bulb.

*Reproductive System.*—As a result of the outward resemblance of the reproductive system of the female coccid to that of other insects, past investigators have described it as comparable to the usual insect type. According to Emeis,[7] however, such a comparison, except perhaps in the widest sense, is hardly warranted. Until recently the origin of the three ovarian elements, namely the nurse cells, egg cell, and epithelial cells, was very obscure. Leydig,[17] one of the earliest investigators, working on *Coccus hesperidum*, claimed that the nurse cells, egg cell, and epithelial cells must have arisen from undifferentiated germ glands. Leuckart[16] and Lubbock[19] came to the conclusion that these three elements are all originally undifferentiated cells of the germ rudiment. Emeis[7] failed to show whether the epithelial cells, from which the egg and nurse cells develop, came from the primordial cells or from the original mesoderm.

Shinji,[31] however, in his work on the morphology of the coccids, showed conclusively that the three ovarian elements are derived from the primordial germ cells. According to Shinji, the germ cells in the ovary give rise to a mass of oögonia by means of mitosis. After undergoing a resting period, a few oögonia, situated along the periphery of the ovary, undergo a last division, giving rise to four oöcytes. Shortly after this a protoplasmic substance is secreted toward the proximal end. These three secretory oöcytes nourish the single oöcyte located below, and thus become the so-called nurse cells. The subsequent history of the oögonium or ovariole is described as follows, by Shinji:

> The cytoplasm areas of the fast-growing nurse cells soon come into contact with one another. Being colloidal in nature, the nutritive substances secreted by the nurse cells elongate in the direction of the least resistance, which is in this case toward the egg nucleus. . . . The nutritive substance, which is elaborated by the nurse cells, literally pours over the egg, causing a rapid increase in its size. Epithelial cells which surround the nurse chamber above never multiply, but those around the egg multiply rapidly and help to accommodate the protoplasmic substance which pours over the nurse chamber above. Soon a constriction becomes evident at the junction of the two chambers, due partly to the ingrowth of the epithelial cells at the base of the nurse

chamber, and partly to the rapid expansion of the egg and nurse cells. The epithelial cells surrounding the egg chamber are cubical or elongate ovoid in shape, and actively divide, while those surrounding the constriction are smaller and spindle-shaped. No mitosis was observed among the latter.

The brief observation made in the course of the present investigation fully substantiates the findings of Shinji, although he worked upon three species entirely different from *Aonidiella citrina*. It is to his excellent paper that the reader is referred for further cytological and embryological information.

The *female reproductive system* of *Aonidiella citrina* (fig. 9*B*) is situated in the body cavity, ventral to the digestive system, and posterodorsal to the main thoracic ganglion. It consists of a pair of ovaries joined by their respective oviducts to a slender well developed vagina, and forms a figure much the shape of a capital Y. Each ovary carries a large number of ovarioles which are closely and irregularly arranged around the lumen of the oviduct. The ovarioles vary in shape according to their stage of development. In a fully developed ovariole (fig. 9*C*) two regions can be recognized: the first, a proximal chamber containing a large egg cell with its chorion, protoplasm, yolk, and fat cells; the second, a distal chamber containing only three nutritive cells with their large nuclei, and protoplasm of varying density. Since no division takes place in the epithelial cells surrounding the nurse chamber, this layer is appreciably thinner than the corresponding one around the egg chamber, where, as shown before, active cellular division continues. A so-called nutritive cord, starting from a clear protoplasmic center between the nutritive cells, runs through the narrow connecting neck into the egg cell where it spreads in a fanlike manner. Its function, as the name indicates, is for the conveyance of the nutritive protoplasmic substances, essential for the development of the egg. With the growth of the embryo, the contents of the nurse chamber are withdrawn into the egg chamber, with the result that the epithelial layer of the former shrinks to a small mass. When fully developed, the embryo, enveloped in its amniotic covering, passes into the lumen of the oviduct.

The opening of the *seminal receptacle* is found at the junction of the two oviducts (figs. 9*A* and 9*C*). The seminal receptacle is a well developed blind sac which, in the case of fertilized females, is filled with an exceedingly large number of male sperm cells. Located posteriorly of the junction is the vagina, which is a relatively wide canal with its opening on the ventral surface of the body, between the fifth and sixth abdominal segments. Just forward of the opening, the wall of the vagina forms a slight dilatation or uterus. It is probably in the uterus that the

embryo loses its amniotic covering, shortly to appear as a free-moving nymph. Numerous unicellular glands are found around the uterus, while the wall of the vagina itself is of thick intima covered externally by a layer of muscles.

The *male organs* (fig. 10*A*) vary remarkably in structure according to the different metamorphic stages. In this discussion only those of the adult male will be briefly considered.

Occupying the dorso-lateral space of the body cavity and situated above the alimentary canal is a pair of large, ovoid testes. Each testicular lumen is homogeneously filled with a mass of coiled, threadlike spermatozoa. Leading from the testes are the paired vasa deferentia; the latter are short and thick, each having a lumen surrounded by a well developed epithelium. The vasa deferentia unite with a long and slender ejaculatory duct which, after a few convolutions, opens posteriorly into the style. The ejaculatory duct is provided with a chitinous-walled lumen surrounded by a stratum of epithelial cells, which is followed by a coat of muscular tissue. No vesicula seminalis or accessory glands were found to be present.

*Central Nervous System.*—The central nervous system (fig. 11*B*) is comparatively well developed for such an inactive insect as *Aonidiella citrina*. It consists mainly of a cephalic ganglion and a thoracic ganglion or thoracico-abdominal center. The latter is the result of an advanced degree of centralization and fusion of the thoracic and the abdominal ganglia, with the further incorporation of the subessophageal ganglion. No ganglia or nerves representing a sympathetic system were found.

The *cephalic ganglion* (figs. 11*B* and 11*C*) or brain, situated forward of the mouth region, consists of a bilobed body sending out, from its anterior end, two pairs of nerves: the inner pair representing the ocular nerves, and the outer and shorter pair, the antennal nerves. Of the three cerebral regions, the tritocerebrum, projecting downward, is the only one that is clearly distinguished; the other regions, as a result of the loss of eyes and antennae, are rudimentary and difficult to differentiate. From the supero-posterior part of each brain half arises an esophageal nerve commissure which extends backward, passing on the outside of the esophagus, and continues posteriorly, connecting with the thoracic ganglion (figs. 10*D* and 11*C*).

The *thoracic ganglion* (fig. 11*A*) is a prominent, compressed body, situated in the median portion of the body. It consists, as said before, of five pairs of ganglia fused together, representing the subesophageal ganglion, the three thoracic ganglia, and the abdominal ganglion. The

component thoracic and abdominal ganglia each give off lateral nerves which further divide into smaller nerves supplying the body organs.

Surrounding the medullary "Punkt aubstanz" (fig. 10*D*) of the cephalic and thoracic ganglia, there is a well developed cortical layer made up of numerous ganglionic cells. Each ganglinoic cell contains a well defined nucleus which, exclusive of the periphery, takes up the haematoxylin stain very rapidly. The ganglia, as well as the nerves, are further enveloped in a thin, transparent membrane or epineurium.

In regard to visible response to external stimuli, a similar condition as noticed by Childs[3] in *Epidiaspis,* also held true here:

> The only movement that could be noticed was that of the drawing in or telescoping of the posterior region when touched with a needle. This shortening takes place through the contraction of the segments. No other movements of the body were observed in response to other stimulants, such as light, heat, and water.

*Respiratory System.—Aonidiella citrina* possesses a respiratory system more or less characteristic of the entire Diaspine group. The whole system was studied with ease by examining early third-stage females under the microscope. Specimens, unattached to their scale coverings, were mounted opposite a small, solid-black area on a microscopic slide, either in water or glycerin. By this method the silvery-white trachea stood out against the black background. The general arrangement of the tracheal system is shown in figure 11*B*. Only the outstanding trunks and branches are indicated, as the minor branches with their ramifications among the internal organs, form a very complex system, and it would serve no useful purpose to try to represent them here.

The respiratory system (figs. 10*B* and 10*C*) consists of two pairs of stigmatic openings or *spiracles,* placed equidistantly on the ventral surface of the body, well within the margin. The anterior pair of spiracles is called the mesothoracic spiracles, and the posterior pair the metathoracic spiracles. The supplementary dorsal abdominal spiracles (mentioned by Newstead[25] as being present in *Stigmacoccus* and *Perissopneumon* of the Monophlebinae, and by Oguma[26] in *Xylococcus* of the Xylococcinae) do not occur in the Diaspinae. The tracheae are numbered and named, according to the original areas they cover, and to their corresponding homologies in the free-moving nymphs.

Each mesothoracic spiracle forms the opening of a short, wide trunk which immediately branches internally, giving rise to branches *a, 15, b, 2,* and *c.*

Branch *a* divides, giving rise to the ocular (*16*) and foreleg (*17*) tracheae.

Branch *15* covers the antennal area and becomes forked toward its extremity.

Branch *b* divides, forming the anterior (*18*) and the posterior (*19*) tracheae of the mouth region, and also the dorsal anterior trachea (*6*).

Branch *2* traverses the middle of the body and joins its fellow from the other side, thus forming the mesothoracic transverse tracheal trunk. Before this fusion is made, the longitudinal thoracic tracheal trunk (*5*), the original middle leg trachea (*11*), and the ventral thoracic trachea (*1*), are given off. About halfway down trunk *5*, the median lateral trachea (*14*) branches off.

Branch *c* divides, giving rise to the posterior (*12*) and the anterior lateral tracheae (*13*).

Each of the metathoracic spiracles also leads into a short trunk which immediately divides into seven branches. These are:

Branch *5b* passes anteriorly and unites with branch *5a* of the mesothoracic spiracle, thus forming the longitudinal thoracic tracheal trunk.

Branch *10* is the original wing trachea.

Branch *9* is the original hind leg trachea.

Branches *8*, *3*, and *7* form the posterior abdominal tracheae.

Branch *4* (as with branch *2* in the mesothorax) unites with its mate from the other metathoracic spiracle, thus forming the metathoracic transverse tracheal trunk.

The aperture of the spiracle itself is surrounded by a chitinous rim (fig. 10*C*) which is continuous ventrally with what is known as the muscle plate. The opening leads into a chamber called the collar chamber, which extends on each side, forming two small lobes. At the base of the chamber, ridgelike processes were noticed projecting into the lumen. These projections, together with muscles running to the muscle plate, probably constitute a closing apparatus, similar to that which was shown by Savage[29] to be very well developed in *Monophlebus* (Monophlebinae). Although longitudinal and cross sections of the tracheal trunks were examined, in no instance was the presence of taenidial rings detected.

*Dorsal Vessel.*—List,[18] working on the internal anatomy of *Orthezia cataphracta*, is perhaps the only worker who has definitely described a dorsal vessel for a member of the Coccidae. He writes:

Bei Herauspräparation des Darmes und der Malpighi'schen Gefasze gelang es mir, einen aus zarten Wanden bestehenden Schlauch, der an der auzeren Membranen der Malpighi'schen Gefasze haftete, zu beobacten. Er lag auf der dorsalen Seite derselben, hatte eine bedeutende Lange und musste, so viel ich aus derselben schatzen konnte, bis in der vorderen Theil des Thorax reichen.

The presence of such a complete organ of circulation in the Coccidae has subsequently been doubted by Oguma[26] and other investigators, with the result that at present the Coccidae are quoted as not possessing an organ comparable to the dorsal vessel in other insects. Blood circulation in this group is described as taking place by the contraction and expansion of certain body cavities, especially the pericardial sinus.

After a thorough study was made of all the internal organs, the writer became fully convinced that *Aonidiella citrina* does possess a dorsal vessel (fig. 12*A*). A highly nucleated duct is found running from the caudal extremity of the body, through the thorax, and into the head where it terminates in the region of the cephalic ganglion. It follows throughout the median dorsal line just beneath the integument. Longitudinal and cross sections through the stomach show the dorsal vessel to be in close association with the outer wall of the latter. Posterior to this portion it widens appreciably to form the heart, after which it tapers down to a fine tube toward both the abdominal and cephalic regions.

Because of the delicate nature and small size of the dorsal vessel, not much could be learned about the details of its structure, or of the absence or presence of ostia and aortic valves. However, its walls are decidedly cellular, the cells showing a large number of nuclei regularly arranged around a fairly distinct lumen which is further surrounded by a more or less granular protoplasm. The cells are covered externally by a muscular layer, the fibers of which are extremely hard to detect.

Additional evidence as to the presence of a dorsal vessel was brought out by the direct examination of live specimens under the microscope. Studied from a dorsal aspect, a steady and regular pulsation was noticed dorsad of the stomach and at the same position that the dorsal vessel was located in the stained sections.

*Glands.*—The glands of *Aonidiella citrina* are of four types: salivary, wax, pygidial, and esophageal.

The *salivary glands* (fig. 12*B*) are paired, tubular, lobelike structures situated on each side of the fore intestine, and occupying practically the whole of the lateral parts of the mesothorax. Each gland consists of numerous glandular cells arranged radially around branched ducts running from the lobelike structures. The cells or acini are surrounded by a thick layer of granular cytoplasm in which are imbedded numerous large nuclei. The branched ducts open into a common median duct which pursues an inward and forward course. When in close proximity to the mouth region, this median duct turns inward, uniting with another from the opposite corresponding gland, to form a common outlet, opening into the side of the pumping apparatus. Throughout its whole

length, the duct exhibits a narrow but distinct lumen which is lined with a thick, chitinous intima.

Because of their variation in form, size, and location, the *wax glands* (fig. 12*D*) play an important part in the classification of the Coccidae. In the generalized or unarmored coccids, like *Lecanium*, *Xylococcus*, or *Orthezia*, the openings of these glands are scattered over the whole cuticula of the body. In the specialized or armored coccids, the openings are situated in more definite groups toward the posterior end. They may be limited to the lateral halves of the pygidium, where their openings are more or less arranged in three marginal lines on the dorsum (figs. 2*A* and 2*C*), as in *Aonidiella citrina*, or an additional group may be found around the genital opening, the latter being known as the circumgenital glands.

Figure 12*D* shows the structure of one of these wax glands. Each opening forms the outlet to an extremely long and slender invaginated tube, known as the ceratuba. At its anterior extremity, each ceratuba is connected to a highly chitinized, perforated, knoblike structure, which forms a reservoir for the reception of the substances secreted by the central wax gland and the two lateral or accessory glands. The central wax gland consists of an anterior apical, and a posterior cylindrical region, the two being connected by a relatively short stem or neck. Both these regions stand in conjunction with a narrow duct, which, running posteriorly, opens into the chitinous reservoir. The apical region is typically glandular in structure, containing a large nucleus; the posterior cylindrical region, in addition to being glandular, possesses a layer of cells arranged around the common duct, similar to those found in the salivary glands. The paired, unicellular accessory glands which open into the chitinous chamber, on each side of the wax gland opening, differ somewhat from the latter, both in shape and structure. They are more ovoidal and each contains a smaller, though clearly visible, nucleus toward the distal end. Their protoplasmic masses, moreover, are more homogeneous throughout, hardly showing any vacuoles or glandular intrusions. The outstanding difference, however, is their peculiar lumens which, as a result of five or six projecting processes, take on a branchlike appearance. Each lumen tapers posteriorly to form a short, excretory duct which opens into the reservoir.

According to Berlese and Leonardi,[1] the accessory glands secrete a varnishlike substance, which forms a protective layer around the wax thread as the latter is produced by the central wax gland. This theory is quite plausible, as the hard texture and the polished appearance of the female scale covering is probably due to this varnish.

The *pygidial glands* (fig. 12*C*), so called because of their similarity to those of other insects, are paired organs situated posteriorly on each side of the hind intestine. Their respective ducts unite to open shortly in close association with the anus. Each gland is composed of many multicellular lobelike bodies surrounding a poorly defined internal duct. The function of the pygidial glands is not known, but it is very probable that they are also for wax production.

The *esophageal glands,* as pointed out in connection with the structure of the esophagus, are present as small, glandular cells imbedded in the epithelium of the latter.

## SUMMARY

The yellow scale, *Aonidiella citrina* (Coq.), is here recognized as a distinct species and not as a variety of the red scale, *Aonidiella aurantii* (Mask.). This segregation is made as a result of evidence based upon a comparative study of their ecology, biology, and morphology.

Although not well defined in their distribution, the yellow scale seems to prefer the warmer valleys and foothills of the interior, while the red is more abundant closer to the coast.

The red scale attacks all parts of the host tree, whereas the yellow is limited almost entirely to the leaves and fruit.

Reared under similar conditions, the yellow scale completes its life cycle in about 65 days and the red scale in about 60 days, giving an approximate difference of 5 days.

Structural differences are noticed in the pygidial fringes of the two scales. Apart from possessing slenderer lobes and minor variations in the median plates, the pygidium of the yellow scale shows the presence of a fourth lobelike process. Differences in color and texture of the scale covering are also noticed.

In addition to a comparative study of the two species a study was made of the anatomy of *Aonidiella citrina*, which is summarized herewith.

The digestive system is relatively short and straight, with the esophagus and ventriculus showing the highest degree of development. In opposition to Berlese's finding, a definite connection is found between the ventriculus and the small intestine. The gastric epithelium consists of large cells which do not project into the lumen of the ventriculus.

The female reproductive system consists of two well developed ovaries connected to each other in the usual Y-shaped manner. Each ovary carries numerous ovarioles in different stages of development. A fairly large seminal receptacle occurs at the point of fusion of the two ovaries.

The male organs are of the usual type, namely, a pair of testes opening into a long style or penis.

The central nervous system is well defined and consists of two main regions, namely, the cephalic and the thoracic ganglia, the latter having been formed as a result of the fusion of the component subesophageal, the thoracic, and the abdominal ganglia. Antennal, ocular, and lateral nerves are found.

The respiratory system is composed of well developed tracheae which ramify through the whole body. Two spiracles are found on the median-ventral surface of the body. The tracheal branches are classified according to their position.

A profusely nucleated dorsal vessel, consisting of a heart and aorta, runs practically the length of the body just below the dorsal surface. A definite pulsation is noticed.

Salivary, wax, pygidial, and esophageal glands are discussed.

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# ABBREVIATIONS USED IN THE FIGURES

abd.g. abdominal ganglion
ac.gl. accessory gland
a.ch. antechamber
ant. antenna
ant.hist. antennal histoblast
ant.n. antennal nerve
ar.inf. arcus inferior
ar.sup. arcus superior

b.sut. basal suture
bu.fr. buccal frame
bu.mscls. buccal muscles

cae. caecum
ca.v. cardial valve
c.ch. collar chamber
ceph.g. cephalic ganglion
ceph.r. cephalic ridge
cet. ceratuba
c.gl. central gland
ch.l. chitinous layer
ch.r. chitinous ridge
c.hyp. corneal hypodermis
cla. clavus
c.ne. connecting neck
co.inf. costa inferior
co.l. cortical layer
con. connection
co.sup. costa superior
c.pls. chitinous plates
cru. crumena
cs. conus
cyl. cylinder
cyt. granular cytoplasm

d.ac.eye dorsal accessory eye
div.mscls. divaricator muscles
dor.v. dorsal vessel
du. duct

e.cl. egg cell
e.du. ejaculatory duct
ep. epimera
epi. epineurium
es. esophagus
es.com. esophageal commissures
es.gl. esophageal gland

f.ep. follicular epithelium
f.leg fore leg

gen.hist. genital histoblast
ger.c. germ cells
gl.c. gland cells
gl.du. glandular duct
gl.o. gland opening
gr. groove

ha. haltere
h.leg hind leg
ht. heart
hyp. hypodermis

i. iris
int.sc. interscutellar band

l. lens
L. lobe
l.hist. leg histoblast
ln. lateral nerve
l.st. linear sternite
lu. lumen

mal.t. Malpighian tube
ma.r. malar ridge
mal.b. Malpighian body (bulb)
m.cav. mouth cavity
m.du. median duct
mes.p. mesothoracic prominence
mes.sp. mesothoracic spiracle
met.sp. metathoracic spiracle
m.leg middle leg
m.pl. muscle plate
mscls. muscles
m.st. mesosternum
m.th. metathorax
m.tub. mouth tubercle

n.cl. nurse cell
nu. nucleus
nu.c. nutritive cord

oc.ap. ocular apophysis
oc.n. ocular nerve
o.du. oviduct
op.n. optic nerve
ovl. ovarioles

p.eye primary eye
pha. pharynx
pln. plunger
p.th. prothorax
pu.s. Punkt substanz
pyg.gld. pygidial gland

r. retina
rect. rectum
ret.mscls. retractro muscles
ros. rostrum
rstls. rostralis

sal.gld. salivary gland
sc. scape
scl. scutellum
scu. scutum
sp. spermatozoa
spi. spiracle
s.rec. seminal receptacle
st. stem or neck
sty. style or penis
subes.g. subesophageal ganglion
te. testis
th.g. thoracic ganglion
Tp. triangular lobelike process
tra. trachea
tri.c. tritocerebrum
tro. trochanter

u.gl. unicellular glands
ut. uterus
uv. uva

v.ac.eye ventral accessory eye
vag. vagina
v.def. vasa deferentia
ventr. ventriculus
vi.r. visual rods

wg. wing
w.hist. wing histoblast
w.res. wax reservoir

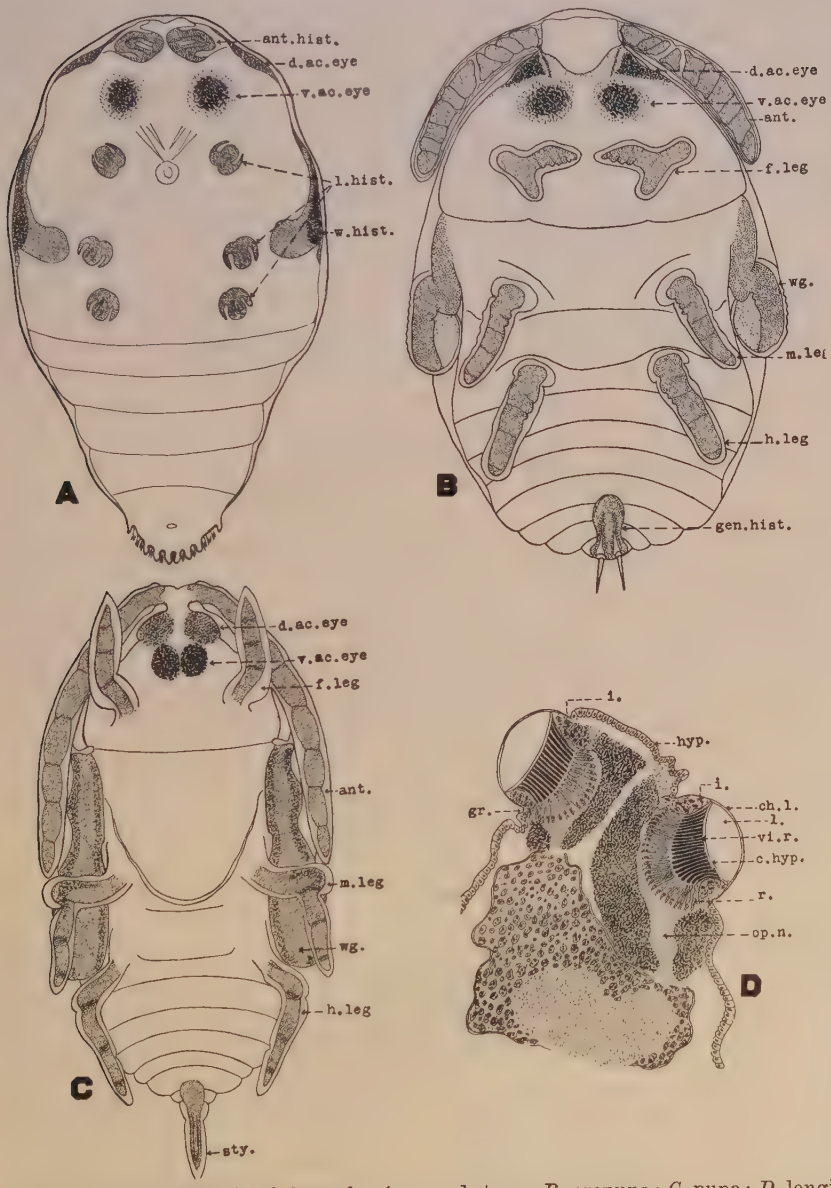


Fig. 1. Metamorphosis of the male. *A*, second stage; *B*, prepupa; *C*, pupa; *D*, longitudinal section through head of an adult male showing structures of dorsal and ventral accessory eyes. Explanation of abbreviations used in this and succeeding figures will be found on page 456.

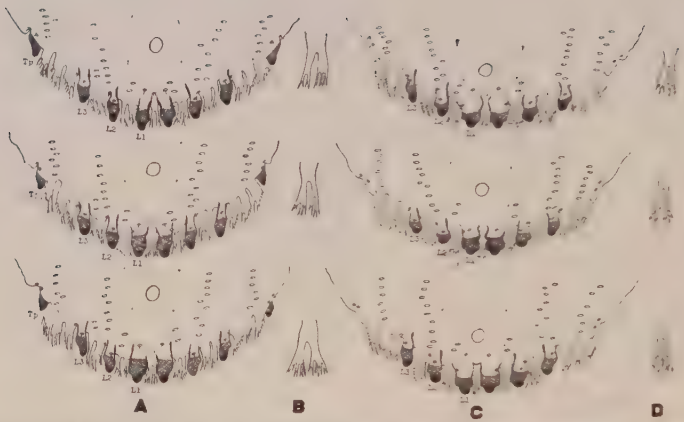


Fig. 2. *A*, pygidia of yellow scale, showing fourth lobelike process; *B*, median plates of yellow scale; *C*, pygidia of red scale; *D*, median plates of red scale.

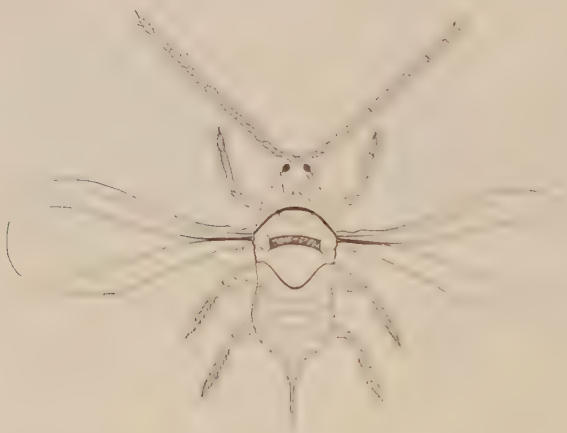

Fig. 3. Adult male of *Aonidiella citrina* (×47).

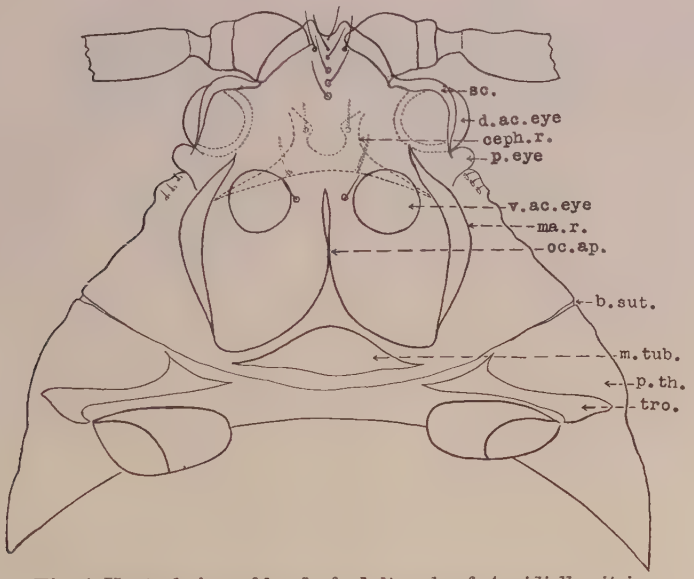


Fig. 4. Ventral view of head of adult male of *Aonidiella citrina.*

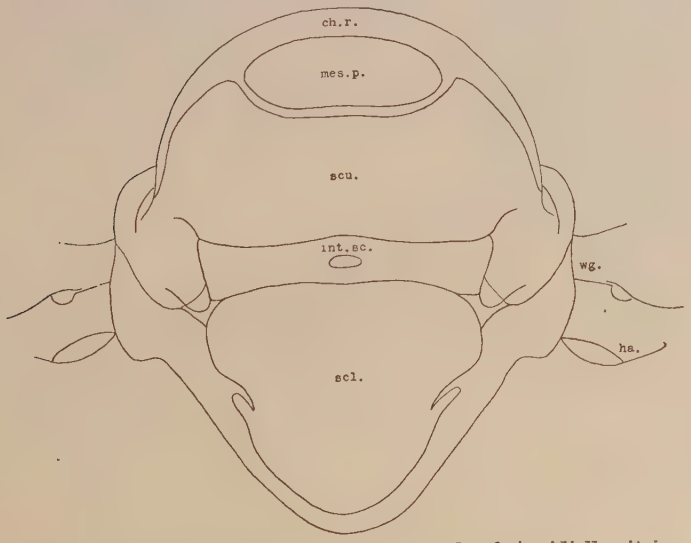


Fig. 5. Dorsal view of mesothorax of adult male of *Aonidiella citrina.*

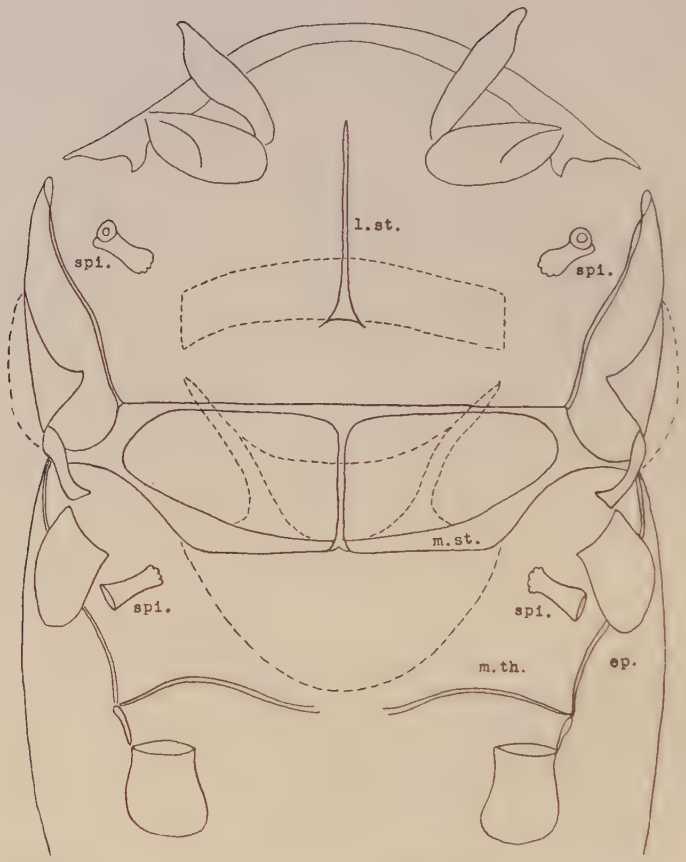


Fig. 6. Ventral view of mesothorax and metathorax of adult male of *Aonidiella citrina.*

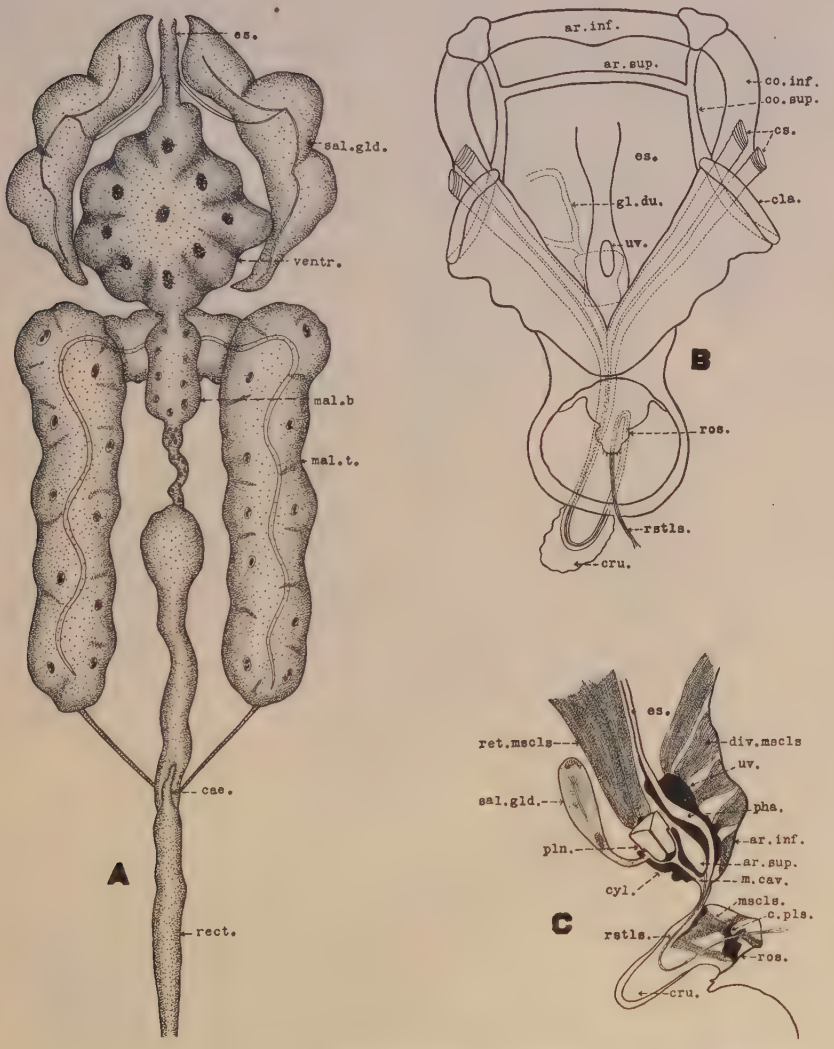


Fig. 7. *A*, digestive system of *Aonidiella citrina; B*, buccal frame (diagrammatic); *C*, longitudinal section of mouth parts.

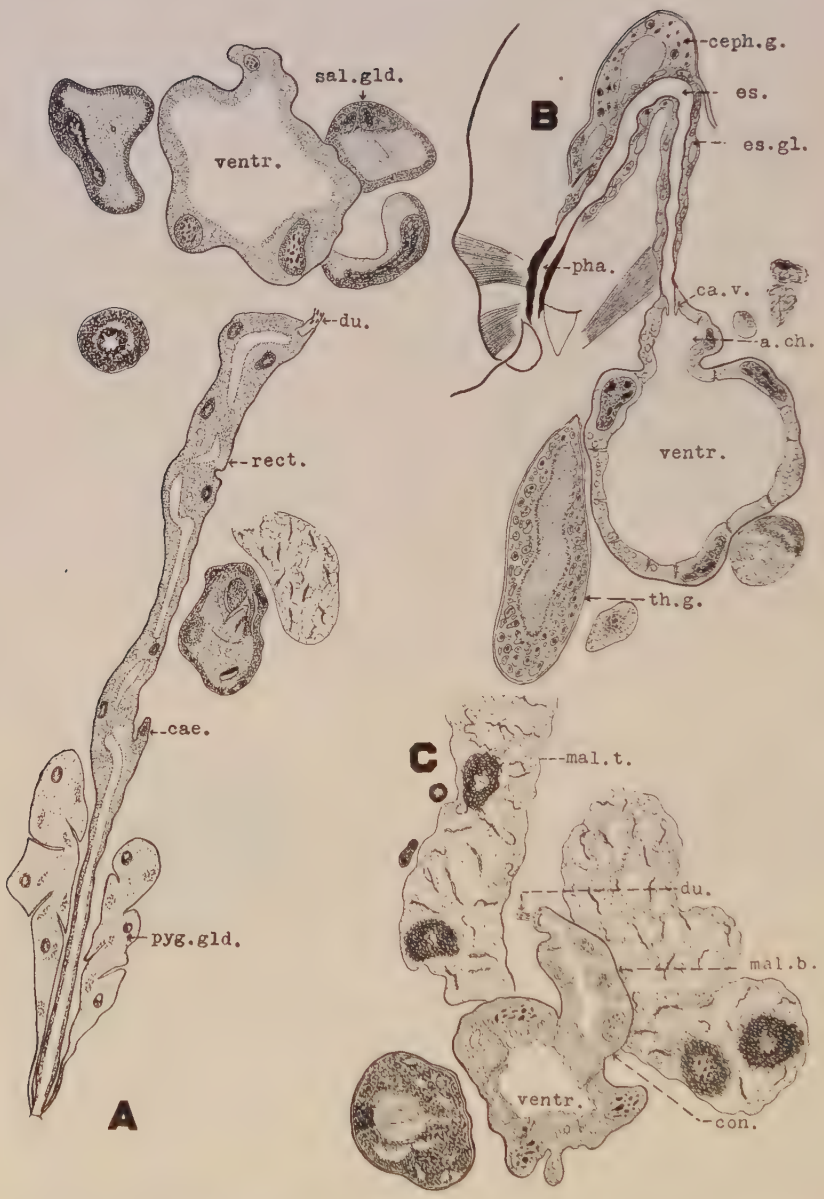


Fig. 8. *A*, horizontal section showing hind intestine; *B*, longitudinal section showing esophagus and ventriculus; *C*, horizontal section showing connection between ventriculus and Malpighian body.

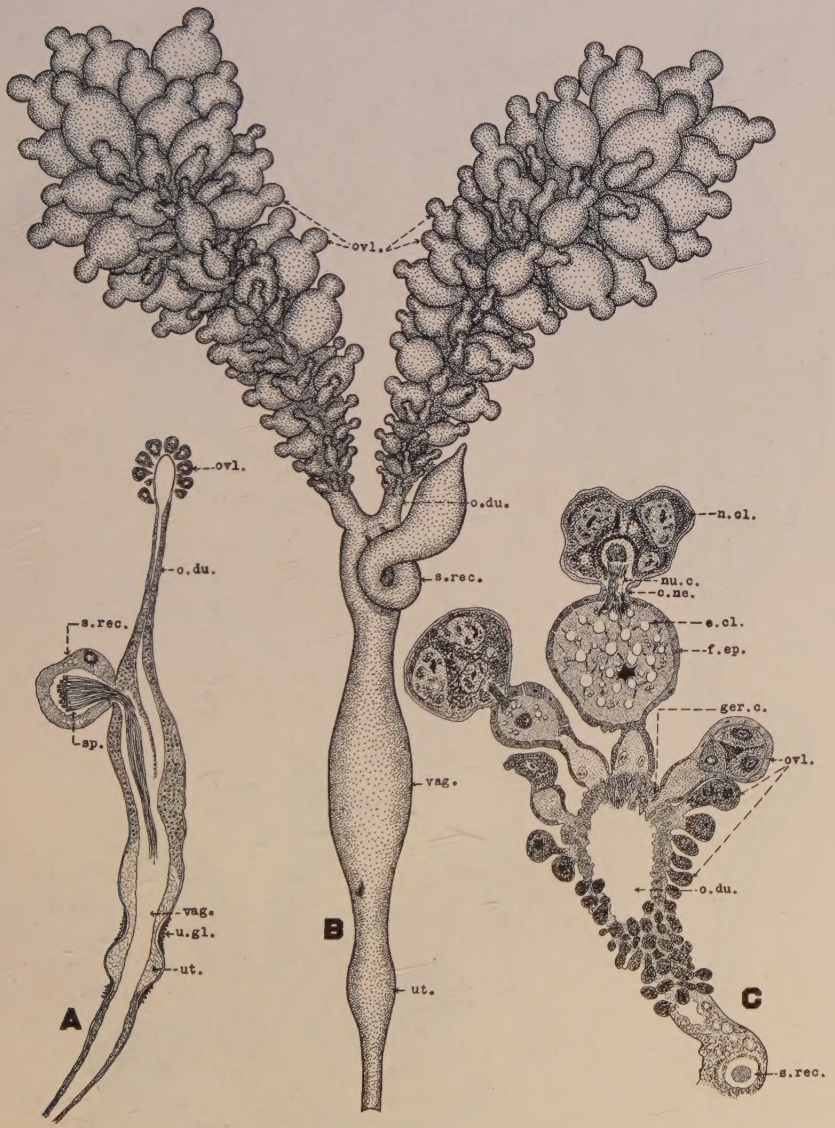


Fig. 9. *A*, longitudinal section of oviduct of *Aonidiella citrina; B*, female reproductive system; *C*, horizontal section through an ovary.

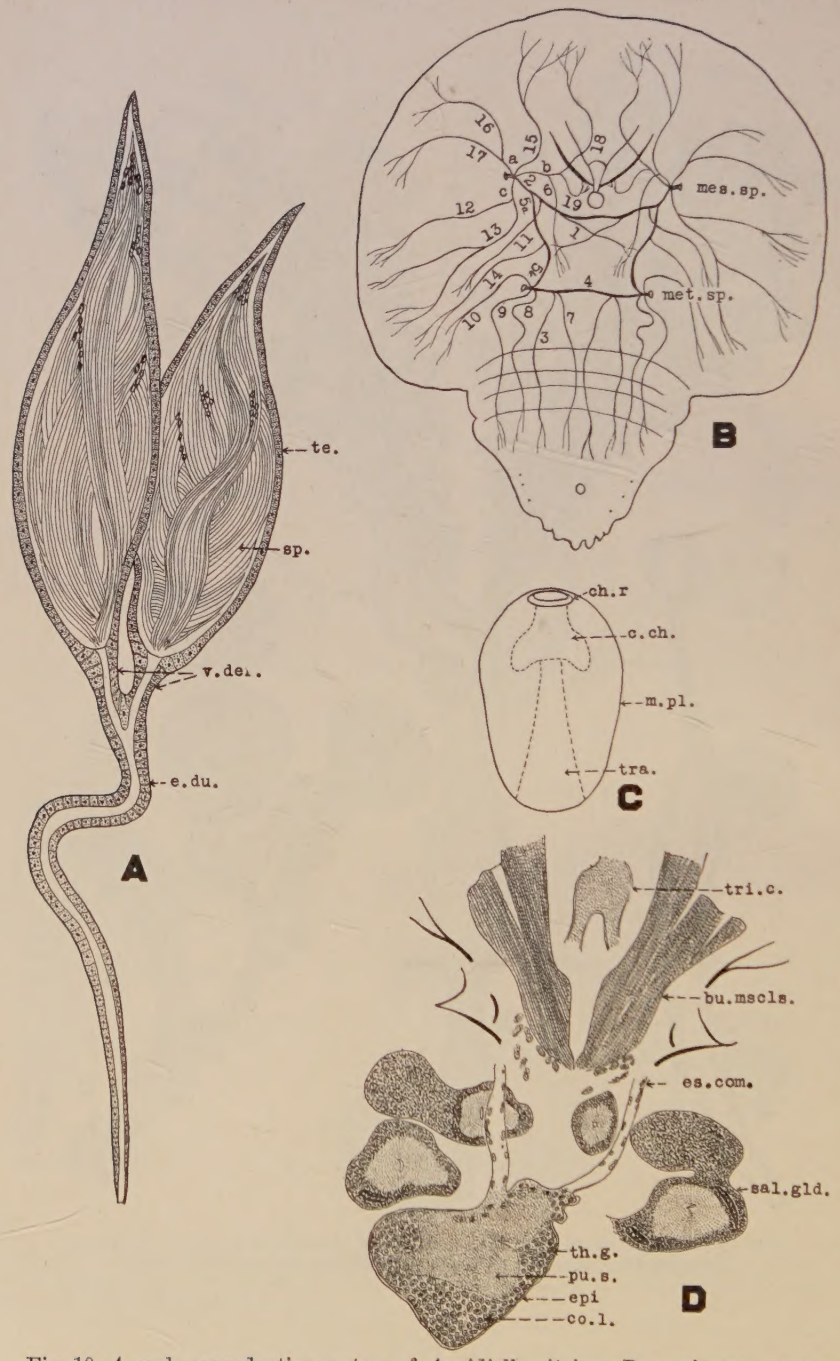


Fig. 10. *A*, male reproductive system of *Aonidiella citrina; B*, respiratory system (semidiagrammatic); *C*, diagrammatic sketch of a spiracle; *D*, horizontal section showing esophageal commissures connecting with thoracic ganglion.

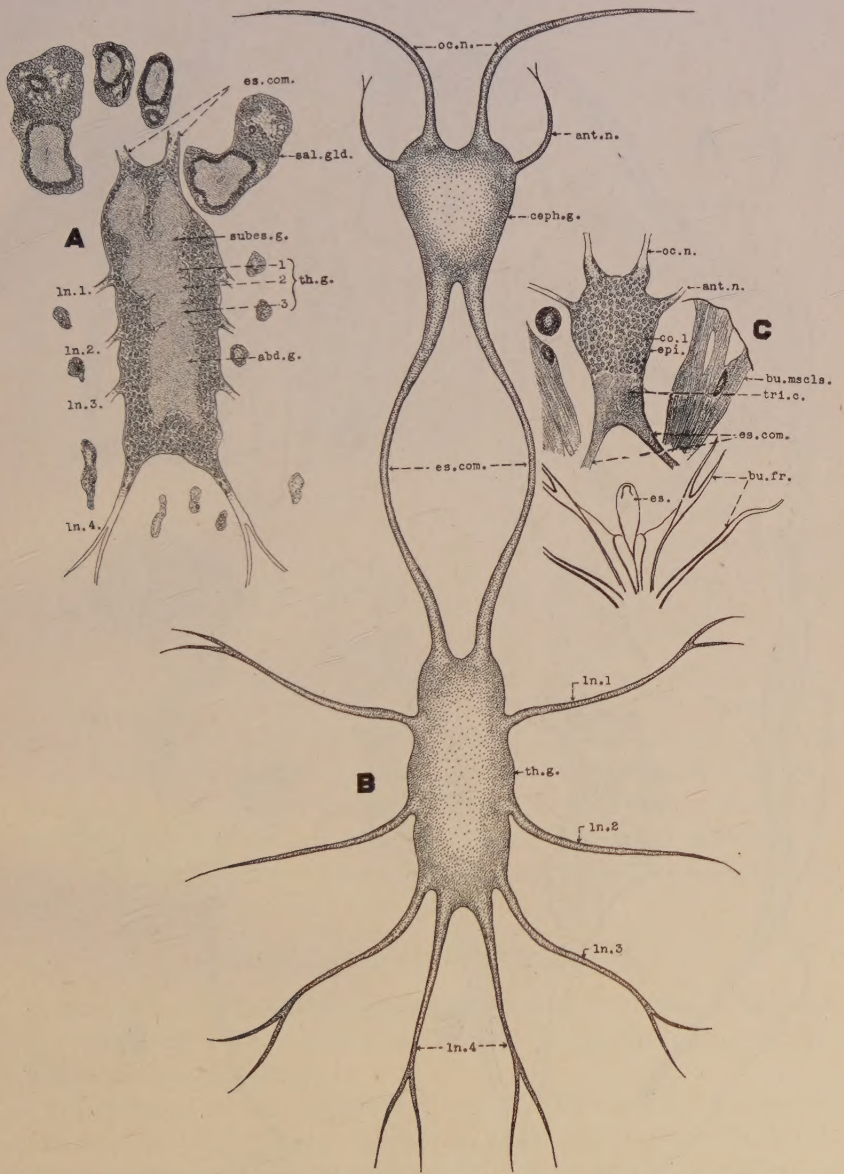


Fig. 11. *A*, horizontal section through thoracic ganglion, showing fusion of subesophageal, thoracic, and abdominal ganglia; central nervous system; *C*, horizontal section through cephalic ganglion.

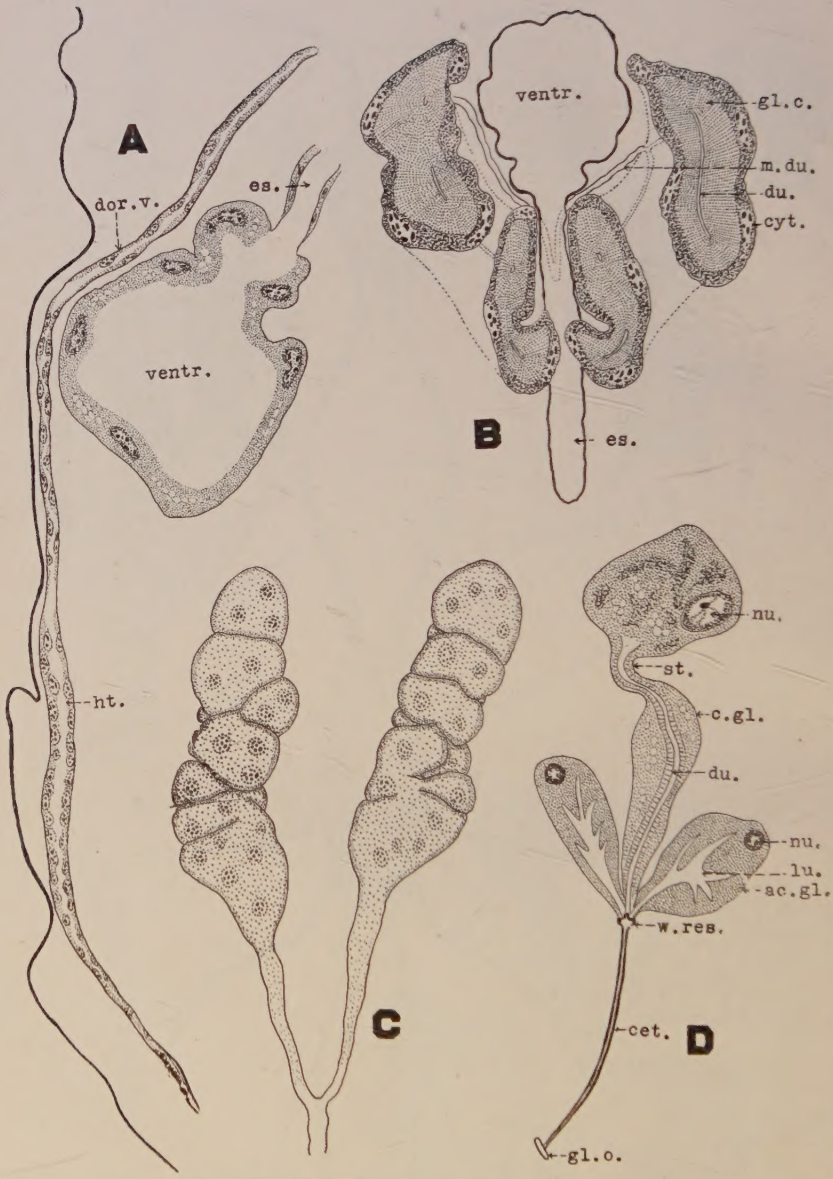


Fig. 12. *A*, longitudinal section showing dorsal vessel and heart; *B*, horizontal section showing salivary glands; *C*, pygidial glands; *D*, a wax gland.